Evaluation healing of jejunal anastomosis in preoperative dexamethasone treated dogs

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Abstract

The objective of this study is to evaluate the healing process of jejunal anastomosis by the aid of histopathology and measurement of bursting pressure of anastomosis site in thirty two adult preoperatively with dexamethasone. The animals were randomly divided into 2 equal groups: Group 1: consists of 16 dogs underwent apposition end-to-end jejunal anastomosis using simple interrupted suture technique which in turn divided into 2 subgroups: subgroup A: consists of 8 dogs treated preoperatively for 15 days with dexamethasone at a dose of (0.2mg/kg) given I/M. Subgroup B: control group consists of 8 dogs not treated with dexamethasone. Group 2: consists of 16 dogs underwent inverted end-to-end jejunal anastomosis using continuous Lembert suture pattern that also divided into 2 subgroups: subgroup A: consists of 8 dogs treated preoperatively for 15 days with dexamethasone at a dose of (0.2mg/kg) given I/M. subgroup B: control group consists of 8 dogs not treated with dexamethasone. The result of bursting pressure measurement showed higher tensile strength in the control groups (445±9.6) in comparison with the steroidal groups (255±25.3) for both techniques. The histopathological study showed that the healing was good in all groups but the rupture that occur due to shedding the pressure lead to non discrimination between which is better in terms of healing. Masson's trichrome showed that collagen content of subgroups taking dexamethasone was lower than that of subgroups not treated with dexamethasone.

تقييم التئام تفمم الصائم في الكلاب المعاملة بالدكساميثازون قبل الجراحة

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الخلاصة

أجريت الدراسة على ٣٢ حيوانا من الكلاب البالغة المحلية تم تقسيمها عشوائيا و بالتساوي الى مجموعتين: المجموعة الاولى: تتالف من ١٦ كلبا اجريت عليها عملية استئصال وتفمم الامعاء باستخدام طريقة نهاية الى نهاية وبتقنية تقابل الطبقات وباستخدام طريقة الخياطة البسيطة المتقطعة وتنقسم هذة المجموعة الى مجموعتين فرعيتين: المجموعة الفرعية A: ضمت ٨ كلاب تم اعطاءها عقار الدكساميثازون لمدة ١٥ يوم قبل العملية الجراحية وبجرعة (٠,٢ ملغرام/كيلوغرام) تعطى عن طريق العضلة. المجموعة الفرعية B: ضمت ٨ كلاب وتعتبر مجموعة سيطرة (بدون اعطاء عقار الدكساميثازون). المجموعة الثانية: تتالف من ١٦ كلبا اجريت عليها عملية استئصال وتفمم الامعاء باستخدام طريقة نهاية الى نهاية وبتقنية الحافات المقلوبة للداخل وباستخدام طريقة الخياطة اللمبرت المستمرة وتنقسم هذة المجموعة الى مجموعتين فرعيتين: المجموعة الفرعية A: ضمت ٨ كلاب تم اعطاءها عقار الدكساميثازون لمدة ١٥ يوم قبل العملية الجراحية وبجرعة (٠,٢ ملغرام/كيلوغرام) تعطى عن طريق العضلة. المجموعة الفرعية B: ضمت ٨ كلاب وتعتبر مجموعة سيطرة (بدون اعطاء عقار الدكساميثازون). اظهرت نتائج قياس قوة الضغط الانفجاري ان قوة الشد في منطقة التفمم كانت عالية في مجاميع السيطرة بينما كانت واطئة في المجاميع التي تم اعطاءها عقار الدكساميثازون وان معدل الضغط الانفجاري كان قليل في كل المجاميع التي

تم اعطاءها عقار الدكساميثازون (٢٥,٣±٢٥٥) ولكن كان عالي في كل مجاميع السيطرة (٩,٦±٤٤٥). كما اظهرت نتائج الدراسة النسجية بان الالتئام كان جيد في كل المجاميع لكن التمزق الذي حصل كان بسبب الضغط المسلط عليه وهذا ادى الى عدم التميز بين ايهما افضل. وكذلك لوحظ خلال الصبغ بالصبغات الخاصة مثل الماسون ثلاثي الكروم بان كل المجاميع الفرعية التي اخذت عقار الدكساميثازون كان معدل تشكيل الالياف الغروية اقل عند مقارنتها بالمجاميع الفرعية الغيرمعاملة بعقار الدكساميثازون.

Introduction

Failure of anastomotic healing was considered as a serious complication of bowel surgery, which increases morbidity and mortality rates significantly (1). The intestinal wall integrity and mechanical strength mostly collagen dependable, which is a structural protein present and concentrated mainly in the submucosal layer (2). Anastomotic healing was mainly assessed by 3parametres; physical evaluation (bursting pressure), histopathological evaluation and biochemical evaluation (tissue hydroxyproline levels) (3). Though the histopathology still the corner stone of studying the healing process, the bursting pressure is an important and dependable mechanical parameter for evaluation of intestinal anastomosis healing (4,5). Corticosteroid is antiinflammatory and immunosuppresive effects. Antiinflammatory effects are complex but primarily occur via inhibition of inflammatory cells and suppression of expression of inflammatory mediators (5). The action of corticosteroids on gastrointestinal tract involves increase in the secretion of gastric acid, pepsin and trypsin they alter the structure of the mucine and decrease mucosal cell proliferation (6). Also usage in gastrointestinal disease to tide the patient over a critical period of the disease in ulcerative colitis and enteritis (7) Mechanisms of action of corticosteroids include; inhibition of the release of arachidonic acid, decrease synthesis of cyclooxygenase-2 (COX-2), inhibition of the production of cytokines, and effect on the concentration, distribution, and function of peripheral leukocytes (8). Despite massive progress in the medical treatment of inflammatory bowel diseases (IBD), corticosteroids still represent the most effective drugs in the management of acute IBD. Unfortunately, surgical intervention under treatment with corticosteroids is often complicated by impaired intestinal wound healing. The aim of our study was to assess the effects of the corticosteroids dexamethasone on intestinal anastomosis in vivo to identify potential causes of impaired intestinal wound healing under corticosteroid.

Materials and methods

Thirty two adult local breed dogs, weighing 15-30 kg, from both sexes, aged from 1.5-3 years were used in this study. The dogs were divided randomly into two groups, sixteen from each group and each group divided into two subgroups, eight for each subgroup. Each animal underwent surgery was fastened from food for 24 hours, 12 hours from the water. Anesthesia induced intramuscularly, by a mixture of xylazine (5 mg/kg) and ketamine hydrochloride (15 mg/kg), and maintained by i.m. administration of increment doses from the same mixture when demanded. The ventral abdominal wall was prepared for aseptic surgery from xyphoid cartilage to umbilical area, a 7-10 cm midline incision was made on the skin in linea alba by scalpel then by blunt dissection with scissors the abdomen was opened. A loop of jejunum was exteriorized through a laparotomy incision with the packing of laparotomy sponges. About 5 cm jejunum loop was selected for resection. Normal saline (Nacl 0.9%) was applied continuously on jejunum out of the abdominal cavity along the time of the operation to avoid dryness, the two ends of the resected jejunum were approximated by using 3-0 polyglycolic acid suture. In group one simple interrupted suture technique (apposition technuique) begin from mesenteric border and the mesentery by simple interrupted suturing. In group two Lumbert's suture pattern (inverted technique) begin from mesenteric border and the mesentery by simple continuous suturing. Then the anastomosis site was checked for leakage by application of gentle pressure on the site of anastomosis, followed by a thorough cleaning of anastomosis site and jejunal loop with normal saline to remove any blood clot before returning it to the abdominal cavity. The abdominal wall incision and skin was closed routinely, and antibiotic spray was applied on the skin incision. Systemic antibiotic, pencillin-streptomycin was injected intramuscularly, daily for 3 to 5 days at a dose rate of 10,000 IU/kg body weight and 10 mg/kg body weight, respectively. Four animals from each subgroup at 7 and 15 days after operation were anesthetized. A piece of 20-25 cm of jejunum including anastomotic site was removed and kept in normal saline for further studies. The mechanical strength of the anastomosis was determined by bursting pressure, which represents the resistance of the jejunum to intraluminal pressure, by using a sphygmomanometer which was modified to be fit for this purpose. Any leakage from the anastomosis site was revealed by the presence of bubbles. The pressure value recorded as leakage pressure was that one which immediately preceded the pressure fall concomitant with the emission of bubbles or disruption of the bowel. Tissue specimens from anastomotic site were collected and fixed in 10% neutral buffered formalin for 48 hours. The specimens were dehydrated, cleared, embedded

in paraffin wax, sectioned at 5 μm thickness and stained with two stains hematoxylin and eosin stain and Masson's Trichrome stains. The results statistically analyzed using ANOVA and Duncan test and the level of significance was at ($P<0.05$).

Results

Bursting pressure measurement

Mean anastomotic bursting pressures are given in table (1). The mean bursting pressure values were lower in all of the steroid treatment groups in comparison with control groups. It has been observed during shedding the pressure on the intestines in steroidal groups for measuring bursting pressures rupture in serous layer but not observed in the control groups. The results showed there was no rupture or leakage from the anastomotic site in the inverted group, but happened away from the site of anastomosis in any area of intact bowel in most of the animals of this group, but in the apposition group, the rupture and leakage was happening in the site of anastomosis in all animals of this group.

Table 1: The mean of bursting pressure ± stander error (S.E.) for both groups after 7 and 15 days

Groups	Apposition of the layers technique by using simple interrupted sutures		Invert technique by use single row of continuous Lumber suture Pattern	
Subgroups	Control group	Steroidal group	Control group	Steroidal group
Mean of bursting pressure at 7 days (mmHg)	440 ± 11.6 A,a	270 ± 30 B,a	370 ± 34.2 C,a	255 ± 25.3 B,a
Mean of bursting pressure at 15 days (mmHg)	425 ± 22.2 B,a	257.5 ± 6.3 C,a	445 ± 9.6 B,b	290 ± 10 C,a

A,B,C the different letter in each row refer to significant differences ($P<0.05$). a,b the different letter in each column refers to significant differences ($P<0.05$).

Histopathology

Histopathological interpretation in the group one (Apposition layers) at 15 days after surgery revealed re-regeneration of epithelial cells of the mucosa and the formation of mature fibrous tissue and infiltration of inflammatory mononuclear cells in the site of anastomosis (Figure 1).

As observed a severe rupture in the mucosal and submucosal layers of the intestine as well as the fibrous tissue (Figure 2). After staining with Masson's Trichrome special stain (M.T.CH) there was of deposition a large amount of collagen fibers in submucosal and serosal layers also in the granulation tissue formed in the site of the anastomosis with the infiltration of mononuclear inflammatory cells and the presence of newly formed blood vessels (Figure 3).
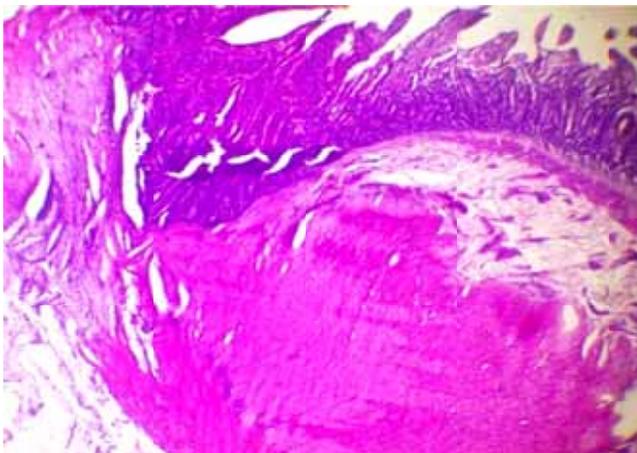

Figure 1: Re-regeneration of epithelial cells with mature fibrous tissue infiltration with mononuclear inflammatory cells and collagen fiber in the site of anastomosis in animal control group after 15 days. H & E stain, X43.
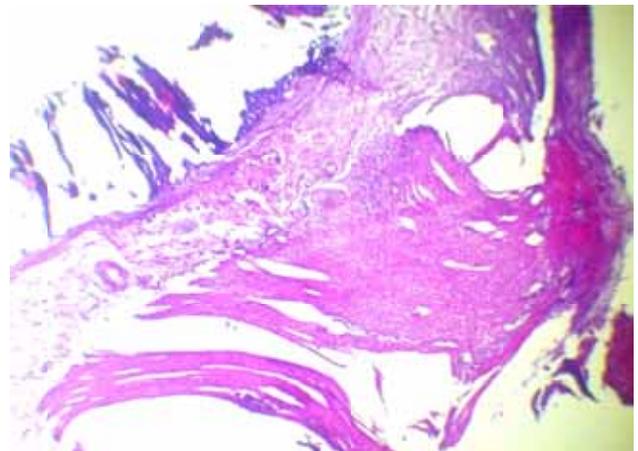

Figure 2: Severe rupture in mucosal and submucosal layers also in fibrous tissue as a result of exposure to severe pressure in animal of dexamethasone group after 15 days. H & E stain, X43.

In group two (inverted technique) there was deposition of dense collagen fibers in the submucosal layer with the granulation tissue formation containing fibers and newly formed blood vessels presence of polymorphonuclear cellsand mononuclear inflammatory cells in the site of anastomosis (Figure 4). As observed re-regeneration of mucosal layers of the intestine and deposition of mature fibrous tissue with infiltration of mononuclear inflammatory cells in the site of anastomosis (Figure 5). Deposition of collagen fibers in submucosal layer and formation of fibrous tissue which extended to serosal layer (Figure 6).
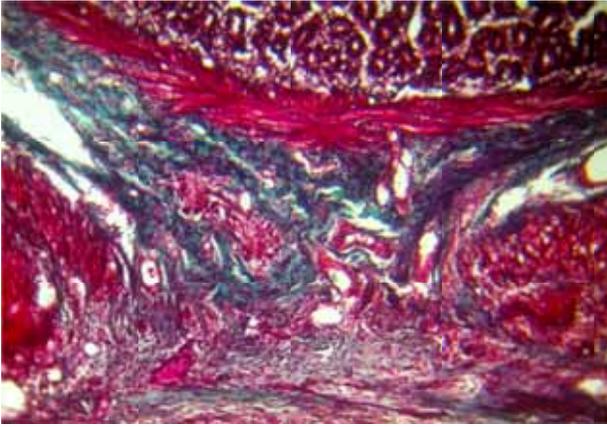

Figure 3: Deposition of collagen fibers in submucosal layer and in granulation tissue in the site of anastomosis and infiltration with mononuclear inflammatory cells in animal of control group after 7 days. M.T.CH stain, X105.
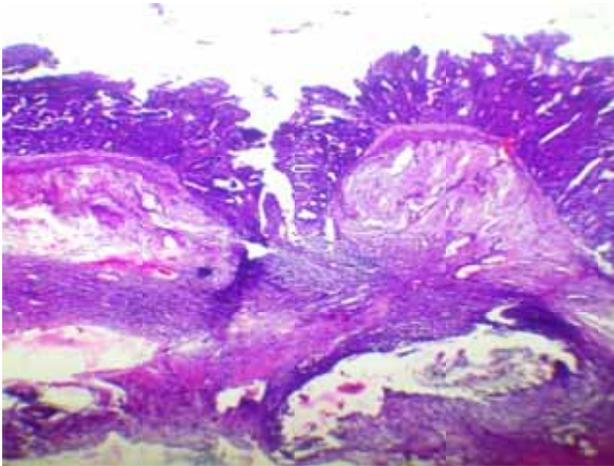

Figure 4: deposition of dense collagen fibers in the submucosal layer with granulation tissue formation infiltrated with polymorphonuclear and mononuclear inflammatory cells in group treated with dexamethasone after 7 days. H & E stain, X43.
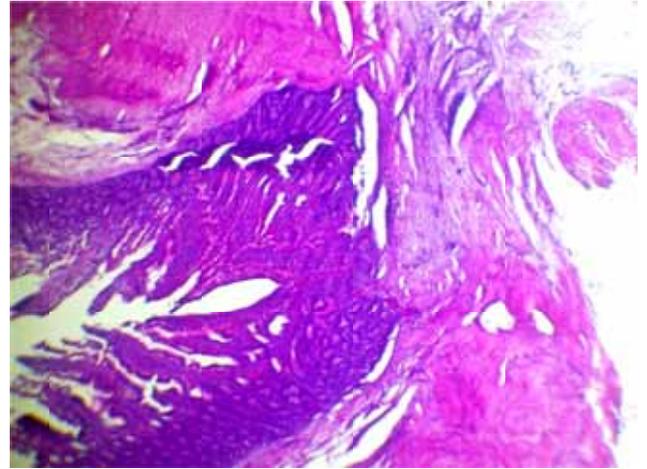

Figure 5: Re-regeneration of mucosal layer of intestine and deposition of mature fibrous tissue with infiltration of mononuclear inflammatory cells in animal of control group after 15 days. H & E stain, X43.
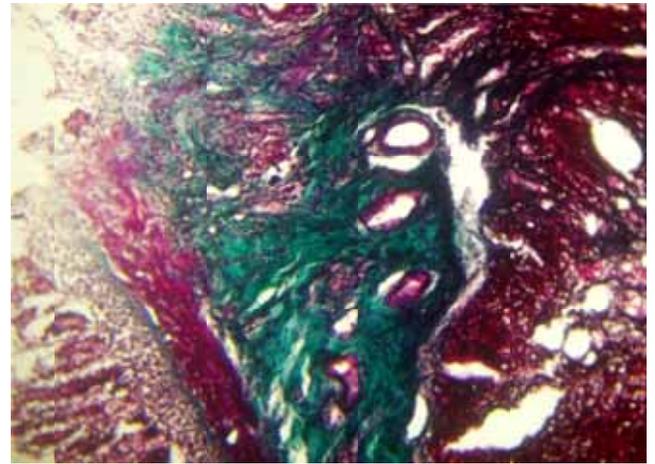

Figure 6: Deposition of collagen fibers in submucosal layer and fibrous tissue formation which extended to serosal layer in dexamethasone group after 15 days. M.T.CH stain, X105.

Discussion

Bursting pressure measurement

That of the methods used to measure the strength of anastomosis site is measuring the strength of the bursting pressure which is a pointer to indicate the efficiency of the healing process (11,12). The results of measuring the bursting pressure in the two groups revealed a significant difference between animals treated with steroid and the animals untreated with steroid in the same group, The mean bursting pressures values were lower in all of the steroid

treatment groups and higher in all control groups and this agree with other authors (9,13,14). Several other studies concluded that only long term steroid treatment significantly weakens colonic anastomosis when use steroid for 60 days (15-17) this disagree with our study when use steroid for 15 days this confirm presence weakens in the site of intestinal anastomosis, confirm previous results on an impaired healing of colonic anastomosis upon corticosteroids treatment (16,18). In the present study, observed the corticosteroids have negative effect on the site of anastomosis and that agree with other authors (9,19-22). One reason for this effect is due to the influence of corticosteroids on the level of hydroxyproline, which contributes to the formation of collagen fiber, where he works on the lower level of hydroxyproline and this decline leads to weakness site the anastomosis and thus has a negative effect on the bursting pressure. This agrees with other authors (23,24) When he proved that level hydroxyproline is low in the steroidal group comparison with the control group. The changes in hydroxyproline content reflect the changes in the amount of collagen (21,25). When measuring the strength of the bursting pressure did not leak or rupture in the site of anastomosis in inverted group, but the rupture was happening away from the site of the anastomosis, in the intact part of the intestines and this result confirms that the anastomosis in this technique will be a force of intact parts of the intestines this agree with (10).

Histopathology examination

The formation of mature fibrous tissue that infiltrated with inflammatory cells and collagen fibers in the site of anastomosis this indicates that access to the maturation phase which is a reorganization of the collagen fiber and remodel. This is consistent with researchers (4,27). The rupture made in the mucous layer returns is caused to the pressure off them when we have a measured bursting pressure, also the slides were stained with special stain was observed that the concentrate of collagen fiber in the dexamethasone group lesser than in the control group was caused returns to the influence of steroidal drugs at the level of hydroxyproline which contributes to the formation of collagen fiber this is consistent with researchers (23,24) also with other researchers (16,18) when they said the use of steroidal drugs in the case of intestinal anastomosis have adverse side effect which include weakness of the wound healing. The mechanism of these effects is to prevent the synthesis of collagen and connective tissue repair (18). The deposition of granulation tissue in all groups refers to the occurrence healing in the first intension and this agrees with finding of other researches (27).

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