Comparison of n-butyl-2-cyanoacrylate tissue adhesive in bladder perforation closure with double-layer suture in a dog model

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This study was carried out at the Surgical Training and Research Laboratory of the Escuela Médico Militar, Lomas de Sotelo, Mexico City, with the backing of the Centro de Biomateriales (BIOMAT) of the Universidad de Habana, Republic of Cuba.

Objective: To compare n-butyl-2-cyanoacrylate with double-layer suture in urinary bladder perforation closure.

Materials and methods: An experimental comparative study was carried out on 30 dogs; 15 in the control group and 15 in the experimental group. Bladder perforation was closed with chromic catgut suture in the control group and with n-butyl-2-cyanoacrylate in the experimental group. Seven variables were analyzed.

Results: There was less time needed for closure and less inflammation with the tissue adhesive and the difference was statistically significant. There was no difference in injury repair usefulness or in clinical recuperation between the two closure techniques.

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Conclusions: In this model, n-butyl-2-cyanoacrylate was equally as safe as double-layer suture with chromic catgut for urinary bladder perforation repair.

Key words: urinary bladder, bladder perforation, cyanoacrylates, Mexico.

INTRODUCTION
The first urological surgical interventions using a cyanoacrylate were experimental and were carried out in 1970 when Aivazian, Kiparisov and Poliakova reported its effectiveness as a tissue adhesive in kidney surgery. Since then various studies have been carried out that report on cyanoacrylate effectiveness in urological surgery.

Sixty percent of bladder lesions are intraperitoneal, 30% are extraperitoneal and 10% are mixed. Extraperitoneal lesions can be treated conservatively with permanent transurethral catheter placement which resolves up to 85% of those cases.

Conventional treatment of intraperitoneal bladder perforation consists of carrying out exploratory laparotomy (or laparoscopic approach) to repair the injury by suturing the bladder with absorbable material, peritoneal wash and permanent bladder drain.

The present study was carried out to look for an alternative technique to bladder perforation suture. The objective was to determine if n-butyl-2-cyanoacrylate (Tisuacryl®) is useful and safe in the repair of this lesion. A dog model was created to compare this tissue adhesive with the 2-layer suture technique. The authors found 2 similar published studies in the literature. The tissue adhesive used in this study was donated to the Surgical Training and Research Laboratory of the Escuela Médico Militar by the Centro de Biomateriales (BIOMAT) of the Universidad de la Habana of the Republic of Cuba.

MATERIALS AND METHODS
The study was an experimental study. All procedures were carried out according to the guidelines described in the NOM-062-ZOO-1999: Technical specifications for the production, care and use of laboratory animals. Permission to carry out the study was granted by the Internal Committee for the Care and Use of Laboratory Animals of the Ethics Committee of the Escuela Médico Militar.

The adhesive Tisuacryl®, n-butyl-2-cyanoacrylate, the colorant gentian violet and polymerization stabilizers were donated to the authors by the Universidad de La Habana, Havana, the Republic of Cuba. Sutures, drugs, curing material, solutions, and food used in the study were paid for by the authors and obtained from different commercial establishments.

The study was carried out with a convenience sample of 30 live, adult, mixed-breed male and female dogs obtained from the animal laboratory of the Surgical Training and Research Laboratory of the Escuela Médico Militar. The animals weighed between 12-25 kg, were between 2-5 years of age, had a healthy aspect and were given antiparasitics and vaccinated.

The dogs were randomly divided into 2 groups of 15 dogs each. The experimental group underwent bladder perforation that was repaired with n-butyl-2-cyanoacrylate. The control group underwent bladder perforation that was repaired with 2-layer interrupted suture using 3-0 caliber chromic catgut.

The study began with a 21-day observational period of the animals in captivity after which each animal from both groups underwent midline incision laparotomy (first surgical stage) under general anesthesia (ketamine and sodium pentobarbital), orotracheal intubation and mechanical ventilation and transurethral urinary drainage with Foley catheter. Two-layer dissection was carried out, exposing the bladder. Animals were hydrated intravenously with 500 mL Hartmann’s solution so that the bladder would fill with urine. Two reference stitches were placed 5 cm from each other at the bladder dome.

On each dog of the experimental group, a horizontal incision 3 cm in length was made with a scalpel in the
bladder dome so that bladder mucosa was exposed and urine leakage was observed. Hemostasis was done by electrocauterization. The incision was held together manually and repaired with n-butyl-2-cyanoacrylate tissue adhesive application. Once it polymerized, wound closure impermeability was confirmed by filling the urinary bladder with isotonic saline solution through a Foley catheter. The bladder was returned to the peritoneal cavity and the abdomen was closed in layers and Foley catheter was removed.

On each dog of the control group, a horizontal incision 3 cm in length was made with a scalpel in the bladder dome so that the bladder mucosa was exposed and urine leakage was observed. Hemostasis was done by electrocauterization. The incision was repaired with double-layer interrupted suture with 3-0 caliber chromic catgut. Wound impermeability was confirmed by filling the urinary bladder with isotonic saline solution through a Foley catheter. The bladder was returned to the peritoneal cavity and the abdomen was closed in layers and Foley catheter was removed.

No food was given to the dogs for 8 hours prior to surgery and 12 hours after surgery. Each animal was given the analgesic flunixin meglumine 1 mg per kg of weight IM every 24 hours for the first 3 postoperative days. Amoxicillin 22 mg per kg of weight was administered IM every 12 hours for the first 5 postoperative days. Nitrofurazone cream at 2% was applied to the wound closure site for the first 10 postoperative days.

Dogs were under surveillance for 21 days after surgery with daily observance of their general state of health, behavior and feeding, defecation and urination habits. During pre- and postoperative surveillance animals were given commercial dried dog food with an energy output of 70-75 Kcal/kg/day.

After 21-day postoperative surveillance each dog underwent a second surgical operation under general anesthesia (ketamine and sodium pentobarbital, orotracheal intubation and mechanical ventilation) in order to resect the entire urinary bladder.

After obtaining the specimen each animal was euthanized by anesthesia overdose. The surgical specimens were separately fixed in 10% formalin, labeled and sent to the pathology department of the Hospital Central Militar to be processed. Two slices were obtained from each specimen from the incision and repair site. One slice was stained with H & E stain technique and the other with the Masson technique. They were analyzed with an OLYMPUS BX50 optic microscope by a pathologist.

The following variables were evaluated:

1. Weight
2. Vital signs (heart rate, respiratory rate and temperature)
3. Surgery duration (minutes): from the moment of skin incision to final wound suture
4. Inflammation: inflammatory cell count (polymorphonuclear and monomorphonuclear)
5. Fibrosis (collagen fiber at scar site)
6. Hemorrhage: extravasated erythrocyte count
7. Giant cells: count

Results from the suture group and the n-butyl-2-cyanoacrylate group were compared for statistically significant differences.

### RESULTS

The surgical procedure described in the Materials and Methods section was carried out at the Surgical Training and Research Laboratory of the Escuela Médico Militar on a total of 30 dogs that met inclusion criteria. They were divided into 15 dogs in the experimental group and 15 dogs in the control group.

During the 21-day postoperative daily surveillance period in which the general state of health, behavior and feeding, defecation and urination habits of each animal were observed there were no changes indicative of any surgical complications. There were no cases of macroscopic hematuria, infection, seroma, abscess or wound dehiscence.

All animals lived from the first day of postoperative surveillance until they were euthanized. Weight, heart rate, respiratory rate and temperature of each animal were measured in immediate preoperative and postoperative periods and on days 1, 2, 3, 14 and 21 of the postoperative period. Preoperative registers were used as references and were compared with all other registers using the Tukey multiple comparison. All registers were normal and no statistically significant difference was found ($P = 0.05$) between study groups.

Surgery duration of the first procedure was measured by chronometer. All interventions were carried out by the same surgeon. The following time values in each study group ($n=15$) were found when compared using the paired Student $t$ test: minimum value was 40 minutes in the control group (with suture) and 33 minutes in the experimental group (n-butyl-2-cyanoacrylate); maximum value was 65 minutes in the control group and 58 minutes in the experimental group; the mean was 56.43 minutes in the control group and 58.14 minutes in the experimental group. Standard deviation (SD) was 20.43 in the control group and 15.84 in the experimental group. There was statistically significant difference...
between the two groups \((P = 0.0001)\). Surgery duration was shorter in the experimental group (Image 1).

There were no cases of wound dehiscence of the bladder incision repair in either group.

There were a total of 30 specimens and 60 slides stained with H & E and Masson stains.

- **MICROSCOPIC FINDINGS**

Polymorphonuclear cells: Cell count was compared between both groups using the Mann Whitney test: minimum value was 13\% in the control group (with suture) \((n=15)\) and 4\% in the experimental group (n-butyl-2-cyanoacrylate) \((n=15)\); maximum value was 20\% in the control group and 12\% in the experimental group; mean value was 18\% in the control group and 10\% in the experimental group; SD was 8.43 in the control group and 4.19 in the experimental group. There was statistically significant difference between the two groups \((P = 0.0001)\). There was less polymorphonuclear cell infiltration in the experimental group (Image 2).

Lymphocytes: Cell count was compared between groups using the Mann Whitney test: minimum value was 59\% in the control group (with suture) \((n=15)\) and 36\% in the experimental group (n-butyl-2-cyanoacrylate) \((n=15)\); maximum value was 70\% in the control group and 42\% in the experimental group; mean value was 68\% in the control group and 40\% in the experimental group; SD was 31.8 in the control group and 18.76 in the experimental group. There was statistically significant difference between the two groups \((P = 0.0001)\). There was less lymphocyte infiltration in the experimental group (Image 3).

Plasmatic cells: Cell count was compared between groups using the Mann Whitney test: minimum value was 23\% in the control group (with suture) \((n=15)\) and 13\% in the experimental group (n-butyl-2-cyanoacrylate) \((n=15)\); maximum value was 27\% in the control group and 18\% in the experimental group; mean value was 24.30\% in the control group and 16.33\%
in the experimental group; SD was 9.18 in the control group and 7.82 in the experimental group. There was statistically significant difference between the two groups ($P = 0.0001$). There was less plasmatic cell infiltration in the experimental group.

Macrophages: Cell count was compared between groups using the Mann Whitney test: minimum value was 5% in the control group (with suture) (n=15) and 3% in the experimental group (n-butyl-2-cyanoacrylate) (n=15); maximum value was 8% in the control group and 5% in the experimental group; mean value was 6.7% in the control group and 4.8% in the experimental group; SD was 2.85 in the control group and 1.97 in the experimental group. There was statistically significant difference between the two groups ($P = 0.0001$). There was less macrophage infiltration in the experimental group.

Fibrosis: Cell count was compared between groups using the paired Student $t$ test: minimum value was 30% in the control group (with suture) (n=15) and 20% in the experimental group (n-butyl-2-cyanoacrylate) (n=15); maximum value was 50% in the control group and 30% in the experimental group; mean value was 45.43% in the control group and 23.77% in the experimental group; SD was 11.87 in the control group and 7.21 in the experimental group. There was statistically significant difference between the two groups ($P = 0.0001$). There was less fibrosis in the experimental group.

Hemorrhage: Extravasated erythrocyte count was compared between groups using the paired Student $t$ test: minimum value was 9% in the control group (with suture) (n=15) and 0% in the experimental group (n-butyl-2-cyanoacrylate) (n=15); maximum value was 32% in the control group and 7% in the experimental group; mean value was 28.33% in the control group and 40% in the experimental group; SD was 7.61 in the control group and 2.16 in the experimental group. There was statistically significant difference between the two groups ($P = 0.0001$). There was less hemorrhage in the experimental group.

Giant cells: Cell count was compared between groups using the Mann Whitney test: minimum value was 3% in the control group (with suture) (n=15) and 2% in the experimental group (n-butyl-2-cyanoacrylate) (n=15); maximum value was 5% in the control group and 4% in the experimental group; mean value was 3.9% in the control group and 2.3% in the experimental group; SD was 1.48 in the control group and 1.19 in the experimental group. There was statistically significant difference between the two groups ($P = 0.0001$). There was less giant cell infiltration in the control group.

### DISCUSSION

Bladder injury can be produced by blunt or penetrating trauma. Bladder rupture from blunt trauma is more common and its usual cause is motor vehicle accident. Penetrating trauma is usually the result of sharp object or firearm injury. Traumatic bladder perforation is more common in men than in women. Iatrogenic bladder injury is more common in women and usually occurs during gynecological procedures.

Bladder traumatism is classified as contusion, rupture and perforation. Bladder contusion is characterized by the appearance of ecchymosis, submucosal hematoma and mucosal tear. They are the most frequent lesions and generally progress spontaneously until they are completely healed without consequences by means of bladder catheter for 7 or 8 days. Bladder rupture due to overextension is typical of traumatism or accidental falls in drunken patients with full urinary bladder. Bladder perforation can be extraperitoneal, intraperitoneal or mixed.

Extraperitoneal lesions can be treated conservatively by placing transurethral catheter for 10 days and is sufficient in 85% of cases. In contrast, all intraperitoneal lesions should be surgically examined.

Intraperitoneal bladder perforation occurs in a completely distended bladder due to a sudden increase in intravesical pressure in abdominal trauma resulting in damage to the bladder dome which is the weakest part of the bladder.

Many small bladder lesions are caused by the Veress needle during port insertion in laparoscopic surgery or during procedures of bladder neck suspension. They can sometimes be managed conservatively by permanent drainage with bladder catheter.

Bladder perforation diagnosis should be suspected from clinical symptoms. Bladder perforation diagnosis is confirmed through urethrocytography (85-100% sensitivity) and is carried out only if urethral rupture has been ruled out. Contrast material extravasation limited to the pelvis is observed in extraperitoneal perforation. In intraperitoneal perforation contrast material appears diffuse in the peritoneal cavity and can be accumulated in the parietal colic recesses or the subdiaphragmatic space. Intravenous urography has 15% sensitivity in bladder perforation diagnosis. Computed tomography (CT) is of little diagnostic value but when done with cystography it provides information as to rupture size and location.23,24

During surgical exploration bladder perforation should be confirmed by bladder irrigation by means of urethral catheter and observation of liquid leakage.
Bladder defects can be repaired with two-layer absorbable suture after which the bladder is filled again to verify whether or not there is leakage. Cases with no complications are usually fully resolved.\(^{22}\)

Tissue adhesives are substances that polymerize on contact with tissue and thus are resistant to separation. The ideal tissue adhesive should be strong, biocompatible, safe and biodegradable. The two principal groups of tissue adhesive are those derived from cyanoacrylate and those that are fibrin glues in addition to some semisynthetic compounds.\(^{25,26}\)

Adhesives derived from cyanoacrylate are very strong. They form an impermeable barrier after polymerization and have bacteriostatic effects.\(^{27}\) They began to be used in medicine in the middle of the past century but initially they were extremely toxic to live tissue and this toxicity was inversely proportional to the length of its lateral chain. Since then and up to the present, different compounds derived from the originals have been developed. The most widely used are n-butyl and n-octyl cyanoacrylates. Both have been tested in many medical functions but they have only been formally approved for skin wounds and embolotherapy. N-octyl cyanoacrylate has been shown to have a greater adhesive effect and less tissue reactivity. However, n-butyl cyanoacrylate has been useful in various types of application.\(^{26,28-31}\)

Tisuacryl\(^{\circ}\) is a compound based on n-butyl-2-cyanoacrylate that was developed in Cuba and has been tested in different medical uses with favorable results.\(^{32}\)

In the present study, registered weights were compared in the pre- and postoperative periods to see if there was any indication of surgical complication. No statistically significant difference was found. However, a tendency to gain weight was observed during the days prior to the second operation that was similar in both groups and was attributed to controlled feeding of the animals and their being in a sedentary state. For the same purpose vital signs (heart rate, respiratory rate and temperature) were measured and compared and no statistically significant differences were found.

Bladder function was evaluated by observing each animal’s urination habits and no alterations were observed. Eating and defecation habits were normal in all animals of both groups at 24 hours from the first laparotomy up to the second operation. Although these variables were not contemplated beforehand, there were no cases of dehiscence, seroma, abscess or wound infection.

This study had originally been designed differently. The first difference was in regard to permanent transurethral drainage catheter placement in each dog for 10 postoperative days. However, the animals removed the catheter within the first 24 hours after recovering from anesthesia and so the study design was changed and catheter was no longer placed. It was thought that the adhesive would not resist the tension of a full bladder but such was not the case. This was transitorily confirmed intraoperatively in the first surgery when the bladder was filled with saline solution by means of a transurethral catheter for testing closure impermeability. The second difference was that originally it had been planned to resect only that part of the bladder dome where the incision and repair had been made. But in the second surgery no macroscopic scar was able to be observed in the experimental group. In some cases when specimens were observed under microscope it was seen that the wrong sections had been taken and so the decision was made to compare in the treatments the present study coincides with Orozco-Razón, Pineros Fernández and Maw,\(^{33-36}\) who reported shorter surgery duration with tissue adhesive than with suture, since suture requires more maneuvering. Surgery duration reduction is one of the principles in surgery damage control.\(^{36}\) Here it is applicable to traumatic bladder perforation. Surgery damage control should be carried out through correct evaluation of each case, carrying out simple surgical techniques in the shortest time possible for the purpose of stabilizing a trauma patient. Shorter surgery duration can also be beneficial in emergency bladder perforation surgery of different etiology. Cañizares et al.\(^{37}\) insist that experience is required in applying this adhesive. However, the present study confirms that despite limited experience surgery duration was shorter in the experimental group. It is the personal opinion of the authors of the present study that the repair technique is simpler with the tissue adhesive.

The most important finding in this study was that tissue adhesive as well as suture showed the same usefulness and safety for the repair of bladder injury provoked in this model since no dehiscence was found in the repairs. The study objective was to compare both techniques and group results were identical in this aspect.

The absence of macroscopic scar with n-butyl-2-cyanoacrylate coincides with the majority of reports published on the adhesive’s aesthetic benefits. However, since this observation is not relevant to organ repair, the result was extrapolated to its functional benefit which implies an almost undamaged bladder wall after procedure recuperation. Macroscopic scar was nearly non-existent in the experimental group and there was no evidence of damage in the bladder wall.
Histological study showed greater inflammatory reaction in the control group, possibly due to the fact that the foreign body 3-0 caliber chromic catgut suture is an organic material. There were also more extravasated erythrocytes in the control group, probably due to tissue trauma caused by the suture needles.

The finding of giant cells in the experimental group tissue concurs with that reported by Pelissier, who insists that they contain cyanoacrylate residue. However, despite the presence of these cells there was still less inflammatory reaction in the experimental group of the present study.

Upon beginning the present study, the authors found two similar published reports in which Marcovich et al. and Seifman et al. repaired cystostomy in pigs (8 animals in one study and 4 in the other) with 2-octyl-cyanoacrylate. The main difference in the results of those 2 studies with the present one was that those authors found no difference in the histopathological findings between the adhesive and suture: both techniques caused the same inflammatory changes.

Bladder injury is classified in accordance with the graded injury scale established by the American Association for Surgery of Trauma/Organ Injury Scale (AAST-OIS). Reproduction of a grade III AAST-OIS injury was the goal in the present study (Table 1).

In addition, the Penetrating Abdominal Trauma Index assigns a risk factor from 1-5 for each intra-abdominal organ. This number is multiplied by a second factor based on the severity of the injury. The sum of the scores in the Abdominal Trauma Index (ATI) is correlated to complications such as abdominal sepsis, abscess, fistulas, wound infection, etc. The ATI is very useful in preventing complications in trauma patients undergoing laparotomy but it does not include other factors such as age and associated injuries. Patients with an ATI score above 25 have a possibility above 50% of developing septic complications.

The ATI score of the present experimental models was 2 (obtained by entering the necessary experimental model data at the website http://www.medalreg.com/qhc/medal/ch29/29_11/29-11-ver9.php3#result). The results for this model corresponded to slight penetrating abdominal trauma in which septic complication development probability is low.

Traumatic bladder perforation in this study was carried out with a sharp object (scalpel). Perhaps the experimental technique described can be used in perforations of other etiologies. The present study did not take cost comparison into account. However, it is well-known that the cost of adhesive tissue in Mexico is much higher than that of suture. The cost of each vial of Tisuacryl® when the present study was carried out was $5000.00 Mexican pesos.

This study could serve as a base for carrying out further work on determining the usefulness of other cyanoacrylates, on comparing this adhesive with other cyanoacrylates or with other types of suture (the present study used chromic catgut but other materials can be used to repair bladder perforation), on evaluating long-term histopathological changes and on analyzing the cost/benefit aspect of adhesives.

### CONCLUSIONS

In this study:

1. Tissue adhesive n-butyl-2-cyanoacrylate was equally as useful and safe as interrupted 3-0 chromic catgut suture for bladder perforation repair.
2. Urinary bladder perforation repair was quicker with tissue adhesive n-butyl-2-cyanoacrylate.
BIBLIOGRAPHY


