The Effect of Cyanoacrylic Glue on Rat Testis, Urethra and Spongiosal Tissue: An Experimental Study

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Purpose: We examined the effect of Glubran®2 on the rat urethra and spongiosal tissue as well as the histopathological effect. We also investigated its clinical use.

Materials and Methods: Eight control and 12 study group male Wistar rats were used in our study. The corpus spongiosum tissues, including the urethra and the tunica albuginea of the testis, were cut 0.5 and 0.2 cm long, respectively. Incision areas in the control group were sutured. In the study group Glubran®2 was dripped on the area. Three weeks later all rats were sacrificed. The penile and testicular tissues were histopathologically investigated. One-way ANOVA and the t test were applied for statistical analysis.

Results: When the study group was compared with the control group, the difference in urethral healing ($p = 0.001$), urethral lumen irregularity ($p = 0.036$), spongiosal tissue healing ($p = 0.001$), inflammation ($p = 0.000$), hyperemia-bleeding ($p = 0.036$) and total healing ($p = 0.001$) were statistically significant. When the study and control groups were compared, the difference in inflammation ($p = 0.000$), necrotic areas ($p = 0.002$), tunica albuginea irregularity ($p = 0.005$), Sertoli's and Leydig cell destruction ($p = 0.005$), and testicular spermatogenesis ($p = 0.005$) were statistically significant.

Conclusions: While perfect healing in the urethral and spongiosal tissues supports the clinical application, damage to the seminiferous tubules, decreased spermatogenesis at the operated site, tunica albuginea irregularity in a fourth of the testes and calcification in 1 testis were observed.

Key Words: histoacryl N-blau; glubran 2; testis; urethra; rats, Wistar

Cyanoacrylates are tissue adhesives that form strong connections and rapidly polymerize when the tissue is in contact with water and blood-like environments. The aim of tissue adhesives is to stop bleeding, and accelerate and simplify suturing using traditional polyglactin or other material. In the 1950s it was used for the first time for covering wounds.[1] It contains ethyl, butyl, isobutyl and octyl, and ethyl butyl esters.[2] Butyl or octyl esters are more frequently used for medical purposes.[2]

In medicine today cyanoacrylates are used for priapism, aortocaval fistulas after nephrectomy, erection dysfunction due to veno-occlusive dysfunction, pelvic arteriovenous aneurysms after transurethral prostate resection, circumcision and fistula after the repair of hypospadias as well as in autosomal dominant polycystic kidney disease, vesicourethral anastomosis during open radical prostatectomy, and laparoscopic pyeloplasty in clinical and experimental urology.[2–6] Although side effects and complications have not been reported in clinical use, in a study done with the isobutyl form in rats the development of sarcoma was reported.[5] To our knowledge this study is the first in the literature involving the testis and spongiosal tissue, including the urethra. In our study using a co-polymer of NBCA and the new monomer metacryl oxysulfolane synthesized by the producer, that is Glubran®2, in rats we investigated their effects at a histopathological level on the testis, urethra and spongiosal tissue and, hence, their possible use in surgery of the testis, spongiosal tissue and urethra.

MATERIALS AND METHODS

In our study after obtaining approval from the local ethics committee 20 Wistar male rats weighing between 200 and 250 gm were used. All surgical interventions were performed under sterile conditions by the same team at the same time and environment. The control group was composed of 8 rats and the study group was composed of 12.

The corpus spongiosal tissue of the rats was cut with scissors in layers from distal to proximal to include the urethra from the ventral side and at a length of 0.5 cm (fig. 1). Incision areas in the control group were sutured with 6-zero polyglactin in a single layer. However, in the study group to ensure total coverage Glubran®2 was dripped onto the surface of the cut after the mentioned procedure was repeated by applying an Angiocath™ cannula for a few seconds. The process was terminated after a couple of minutes. A total of 16 testes in the control group and 24 in the study group were used for evaluation. After making a 0.2 cm cut in the tunica albuginea in the control group it was sutured with 6-zero polyglactin. However, in the study group after repeating the same procedure 1 drop of Glubran®2 was applied on

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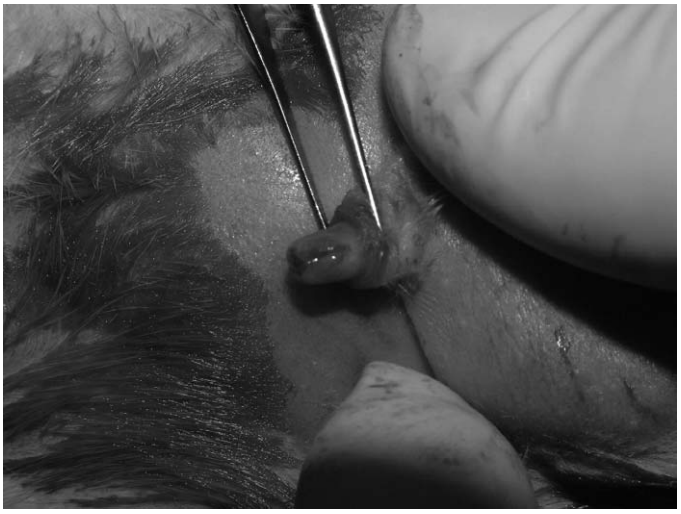

FIG. 1. Urethra and Glubran®2

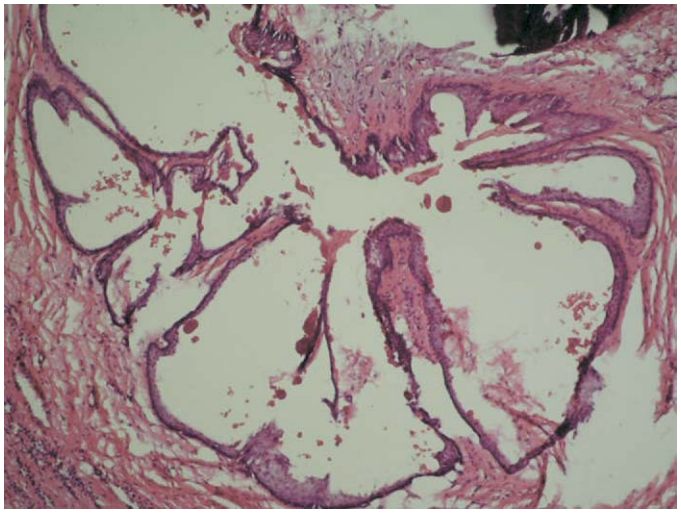

FIG. 3. Control group urethra. H & E, reduced from ×50

the defect area. The process was terminated after a couple of minutes (fig. 2).

Three weeks later all rats were sacrificed by an excessive dose of pentobarbital sodium (100 mg/kg intraperitoneally). The penile tissue and testes were histopathologically examined with hematoxylin and eosin stain by 2 blinded and expertly trained investigators. For statistical analysis 1-way ANOVA and the t test were applied with the SPSS® 11 for Windows® program.

RESULTS

In the control group, an increase in spongiosal material, widespread connective tissue in the urethra, structural irregularity, widespread inflammation and hyperemia-bleeding were present, while widespread areas of necrosis and intense infection were observed during examination of the testis (figs. 3 and 4). However, while normal spongiosal material and urethral results were observed in all except 2 rats in the study group, normal urethras and slight spongiosal material fibrosis were present in 1 (figs. 5 and 6). When the study group was compared with the control group, the differences in urethral healing ($p = 0.001$), urethral lumen irregularity ($p = 0.036$), spongiosal tissue healing ($p = 0.001$), inflammation ($p = 0.000$), hyperemia-bleeding ($p = 0.036$) and total healing ($p = 0.001$) were found to be statistically significant (see table). While tunica albuginea irregularity, widespread inflammation and areas of necrosis were present at operated sites in the control group, spermatogenesis deformation, and partial Sertoli's and Leydig cell deformation were observed. In the study group, while inflammation and necrotic areas were present in 3 testes, in addition to tunica albuginea irregularity, spermatogenesis was also found to be disrupted (fig. 7). Sertoli's and Leydig cells were observed to be normal. Calcification was observed in 1 testis (fig. 8). When the study group was compared with the control group, the differences in inflammation ($p = 0.000$), necrotic areas ($p = 0.002$), tunica albuginea irregularity ($p = 0.005$), Sertoli's and Leydig cell deformation ($p = 0.005$) and spermatogenesis ($p = 0.005$) were statistically significant (see table).

FIG. 2. Testis and Glubran®2

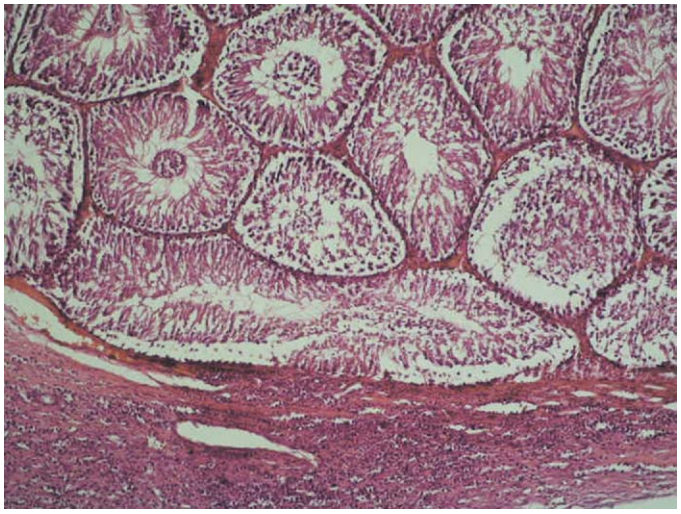

FIG. 4. Necrosis in testis with general inflammation. H & E, reduced from ×80.

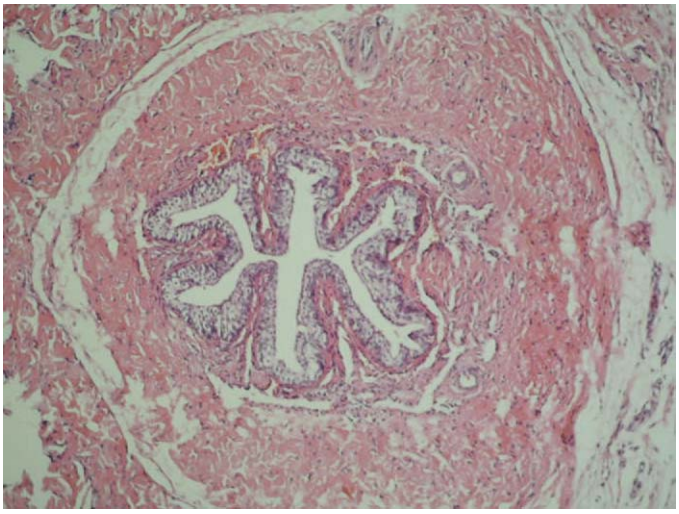

FIG. 5. Study group urethra. H & E, reduced from ×50

Histopathological effect of Glubran®2 on urethra, spongious tissue and testis in rats

	Control Group	Study Group	p Value
Urethra spongiosal tissue			
Overall	8	12	
Urethral healing:			
Good	2	11	0.001
Poor	6	1	
Urethral lumen irregularity:			
Good	3	10	0.036
Poor	5	2	
Spongiosal tissue healing:			
Good	3	11	0.001
Poor	5	1	
Inflammation:			
Present	7	0	
Absent	1	12	0.000
Hyperemia-bleeding:			
Present	5	2	
Absent	3	10	0.036
Total healing:			
Present	2	11	0.001
Absent	6	1	
Testis			
Overall	16	24	
Inflammation:			
Present	14	3	
Absent	2	21	0.000
Necrotic areas:			
Present	9	3	
Absent	7	21	0.002
Tunica albuginea irregularity:			
Present	11	6	
Absent	5	18	0.005
Sertoli's cells:			
Good	5	24	0.005
Poor	11	0	
Leydig cells:			
Good	5	24	0.005
Poor	11	0	
Spermatogenesis:			
Good	5	18	0.005
Poor	11	6	

DISCUSSION

Hemostatics and tissue adhesives, such as sutures, staplers, clamps and biological agents, including thrombin, fibrin, collagen adhesives and synthetic agents such as cyanoacrylates, are used in surgery today.[7] There are a number of advantages of cyanoacrylates. They decrease suture use since fewer sutures can be applied and for smaller incisions no sutures may even be needed, they are rapidly and easily applied, they do not cause pain and their cosmetic effects are excellent.[7] They provide suitable tension, polymerize in moist environments, are biocompatible, undergo gradual resorption and do not cause foreign material reactions.[7] Antibacterial and cytotoxic effects have also been reported.[8] Montanaro et al noted that the toxicity of Glubran®2 is at an acceptable level.[7] It has been reported that Glubran®2 is a surgical adhesive with excellent hemostatic and adhesive properties that forms an effective antiseptic barrier against the contagious and pathogenic substances that may frequently arise during surgery.[9] Although NBCA is known to be antibacterial, in a study Olson et al observed that Staphylococcus epidermidis colonizes on NBCA.[10] In another study investigators noted that surgical adhesives do not inhibit the Niger species of Bacillus subtilis.[7]

Polymerization begins within 1 to 2 seconds after the application of 1 drop to a 1 cm^2 area and the reaction is completed 60 to 90 seconds later. Temperature during the reaction reaches 45C,[9] which is a high but harmless temper-

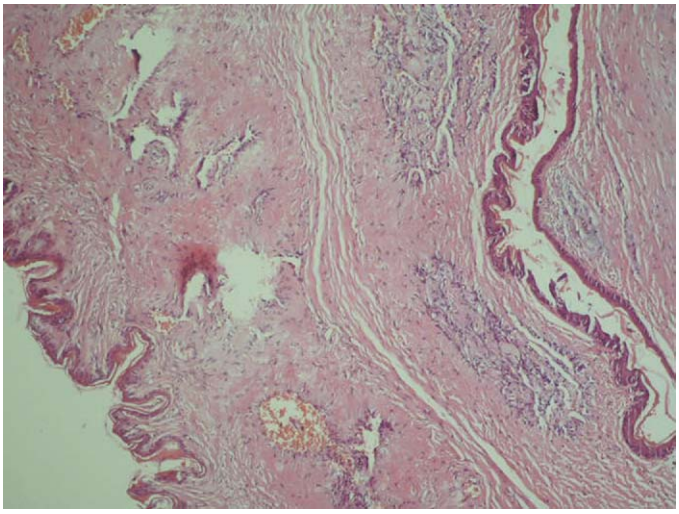

FIG. 6. Mild fibrosis in spongiosal tissue. H & E, reduced from ×50

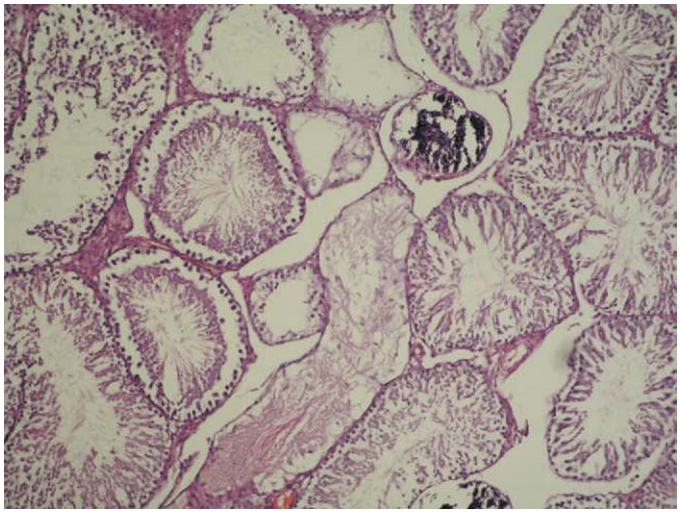

FIG. 7. Hypospermatogenesis and calcification. H & E, reduced from ×80.

FIG. 8. Calcification in testis. H & E, reduced from ×50

ature for the skin. Testicular tissue is especially sensitive to temperature. The body temperature of rats is 36.5C. The testes of rats are located in the lower abdominal cavity. High temperatures cause seminiferous tubule deformation and disrupt spermatogenesis.

In our study the macroscopic results were excellent. However, histopathologically in 6 testes a mixed reaction, irregularity and thickening were present in the tunica albuginea in the region where Glubran®2 was applied. While Leydig and Sertoli's cells remained healthy in approximately 10 seminiferous tubules under the damaged region, generally decreased spermatogenesis and seminiferous tubule damage were observed. While normal structures were noted in all other testes, minimal damage and calcification were present in 1 (see table). In humans the testis is located in the scrotum and the temperature is approximately 1C or 1.5C lower than body temperature. A 45C polymerization temperature in the testis may cause reversible or irreversible damage. In minimal or invasive surgery of the testis the side effects of the drug should be carefully explained, especially to unmarried males or those wishing to father children. More studies, including long-term experimental studies, are required for routine use, also concerning fertility or fecundity.

While cyanoacrylate was initially used in cases of bleeding during radical cystectomy, radical prostatectomy, and anterior and total pelvectomy as well as for lymphorrhea after lymphadenectomy,[11] today they have been successfully used for the treatment of intrarenal pseudoaneursyms[12] after percutaneous nephrolithotomy, renal arteriovenous malformations,[13] fistulas after partial nephrectomy,[3] venogenic impotance,[3] autosomal dominant polycystic kidney diseases,[4] hydrocele,[14] fistulas after hypospadias,[2] rectourethral fistulas[15] and resistant hematuria,[16] and with the aim of preoperative devascularization[17] of paragangliomas in the head and neck. Merimsky and Baratz successfully performed end-to-end urethral anastomosis without sutures in dogs.[18] They reported that no reaction developed and results were excellent. However, Kutlu et al reported a pulmonary embolism after a penile deep dorsal vein embolism in 1 case in which they applied an NBCA and lipiodol mixture.[19] Kim et al applied an NBCA and iodized oil mixture to renal cysts and observed success, that is a 50% decrease in cyst diameter in 81% of the cases.[4] However, this success rate is not a better result than that of treatments with other sclerosing substances.[20] A 62.5% success rate was reported at the 6-month followup of fistulas after hypospadias.[2]

In our study the macroscopic cosmetic appearance of the spongiosal tissue, including the urethra, was excellent. While microscopic urethral healing was excellent, slight fibrosis developed in the spongiosal tissue in 1 case. Short-term results show that it can be used in surgery of the urethra and spongiosal tissue. In addition, the observation that there was no foreign material reaction in the testis or spongiosal tissue was in accordance with findings in other studies in the literature. The long-term consequences of damage observed in 6 testes in a 3-week period require long-term studies. Long-term studies are also required to discover whether any variations occur after the 3-week period in testes with normal findings. Use in testicular surgery can be possible with the results of long-term studies.

As a result, clinically the areas of application of NBCA are increasing. It has hemostatic, antibacterial and long-term adhesive effects, and no damaging cytotoxic and inflammatory effects. It is a product that is easily applied and provides rapid results. However, while excellent healing in the urethra in the short term and excellent cosmetic results were observed, damage to the seminiferous tubules, disrupted spermatogenesis at the operated area, tunica albuginea irregularity in a fourth of the testes and calcification in 1 were observed. However, new long-term studies are required because to our knowledge no information concerning long-term results is available. The effect of Glubran®2 on fertility and fecundity may also be investigated in further studies.

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Abbreviations and Acronyms

NBCA = N-butyl-2-cyanoacrylate

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