Experimental evaluation of 2-octyl-cyanoacrylate on dura mater healing

SUMÁRIO

Objetivo: O objetivo do presente estudo foi avaliar o efeito desta cola tecidual sintética na cicatrização do fechamento da dura mater em coelhos. O estudo foi realizado para investigar o comportamento histológico e toxicológico do reparo dural usando sutura simples e reforçada com adesivo tecidual.

Método: Um total de 54 coelhos foi dividido em 9 grupos de 6 animais, subdivididos em 3 grupos (A, B e C), cada um contendo 3 subgrupos de 6 coelhos, sacrificados aos 7, 30 e 60 dias respectivamente. Após craniectomia parasagital de 1cm e durotomia de 0,5cm, três procedimentos diversos foram realizados: no grupo A dura mater foi reforçada com 2-octyl-cyanoacrilato, no grupo B foi aplicada cola de fibrina e no grupo C foi realizada sutura da abertura dural. A integridade das suturas, existência de abscesso, infecção da ferida e formação de aderência foram documentadas. As cabeças foram enviadas para exame histopatológico.

Resultados: A cola sintética não interferiu no metabolismo dos animais. Os pesos médios de cada grupo aumentaram. As temperaturas não foram estatisticamente diferentes (p=0.210). O processo de cicatrização da duramater, através da atividade fibroblástica e infiltração de células inflamatórias, não diferiram estatisticamente entre os três grupos (p>0.05). Apenas a neovascularização aumentou no grupo controle.

Conclusão: As secções histológicas obtidas da duramater tratada com o polímero 2-octyl-cianoacrilato demonstraram resposta inflamatória mínima, similar a aquelas tratadas com cola de fibrina. Os resultados indicam que esta nova substância parece ter aplicações como um meio auxiliar no fechamento efetivo da duramater.

Palavras-chave: Reparo dural, polímero cianoacrilato, cola de fibrina, histotoxicidade.

ABSTRACT

Objective: The aim of the present study was to evaluate the effect of this synthetic glue tissue on the healing of dura mater closure in rabbits. The study was undertaken to investigate the histological and toxicological behavior of dural repair using standard suture techniques and suture supplemented with tissue adhesive.

Methods: A total of 54 rabbits was divided into 9 groups of 6 animals, and then were divided equivalently into 3 groups (A, B and C), each one containing 3 sub-groups of 6 rabbits, sacrificed on days 7, 30 and 60 respectively. After 1cm parasagittal craniectomy and 0.5cm dural opening, three different procedures were carried out: in group A dura mater was reinforced by using 2-octyl-cyanoacrylate, in group B biological glue (fibrin glue) was applied and in group C only the dura mater suturing was performed. The rabbits were sacrificed on specific days following the operation. Integrity of the sutures, existence of abscess, wound infection and adhesion formation were recorded. The heads were sent for histological examination.

Results: The synthetic glue didn’t interfere with the metabolism of the animals. The mean weight of rabbits in groups A and B increased. Temperature did not increase and was not statistically different between the three groups (p=0.210). Dura mater healing process, as assessed by fibroblast activity and inflammatory cell infiltration, did not differ statistically between the three groups (p>0.05). Only neovascularization increased in the control group. Conclusion: Histological sections obtained from the dura mater treated with 2-octyl-cyanoacrylate polymer demonstrated minimal inflammatory response, similar to that treated with fibrin adhesive sealant. Results indicate that this new substance seems to have applications as an adjunctive means of effecting dural closure.

Key-words: Dural repair, cyanoacrylate polymer, fibrin adhesive sealant, histotoxicity.
INTRODUCTION

Most of cerebrospinal fluid (CSF) leakages are caused by trauma, but they are also one of the post-operative complications of neurological operations.15

Traumatic CSF leakage appears in 2 to 3% of the cranial trauma, 60% occurring in the first days of trauma and 95% within 3 months.25. The incidence of CSF leakage after skull base surgeries is 3.6% in a prospective study with 183 cases.11

The principal consequence associated with CSF leakage is meningitis. This complication happens in approximately 25% of the leaks, of all traumatic types, while including 20% of acute post-operative leaks and 57% of late post-traumatic leaks.7

Dura mater closure following neurosurgical operations can be performed by using sutures. One of the techniques to prevent a CSF leakage is the reinforcement of dura mater suture with glue. Fibrin glue use has led to the successful sealing of CSF leaks. This biological glue has been used in conjunction with sutures to control or stop bleeding, providing fluid and air tightness in many surgical situations.8,12,19,20,22,23,28. The 2-octyl-cyanoacrylate is a topical adhesive that polymerizes to form an adhesive film to hold together the approximated wound edges. The polymerized material is not absorbed by the tissue.9,10. Although it is widely used as a skin adhesive for superficial lacerations, 2-octyl-cyanoacrylate (high viscosity) has not been previously used in experimental dura mater healing, even comparing with and without the application of biological glue.

The aim of the present experimental study was to evaluate the histopathological and toxicological effects of this new synthetic topical adhesive in rabbits, introducing in the neurosurgical practice a cheaper alternative method of repairing and reinforcement of dural suture acting for the prevention and treatment of CSF leakage.

METHODS

Fifty-six white male New Zealand rabbits were used. The rabbits were obtained from the Thomas George bioterio, Laboratory of Pharmaceutical Technology, LTF, Federal University of Paraíba. The study was also approved by the LTF Ethical Committee (numbered 0108/06 on Sep 27, 2006).

All the animals were housed under 21 ± 2°C with unrestricted preoperative access to water and food. The animals that died of anesthetic complication were excluded. Fifty-four rabbits weighing 2 to 4kg survived the entire study period and were randomized into 9 groups of 6 rabbits each. These 9 groups were divided equivalently into 3 groups: group A, in which 2-octyl-cyanoacrylate was applied and the animals were sacrificed on days 7, 30 and 60 following the operation, called respectively A7, A30 and A60 groups, formed by 6 animals each; group B in which biological glue (fibrin glue), was applied and the animals were sacrificed on days 7, 30 and 60 following the operation, called respectively B7, B30 and B60 groups, formed by 6 animals each and, finally, control group, in which only the dura mater suturing was performed and the animals were sacrificed on days 7, 30 and 60 following the operation, called respectively C7, C30 and C60 groups, formed by 6 animals each.

The operations were performed under intravenous thiopental anesthesia and supplemented with additional doses, according to necessity, for maintenance of the anesthetic plan. The rabbits were anesthetized with thiopental 1g in 40ml of 0.9% NaCl solution: 2.5ml of the anesthetic solution was initially applied intravenously, using the access to the marginal vein of the rabbit’s ear. They have been sacrificed after the operation, with an overdose of an application of intravenous thiopental.

Rectal temperature of the animals was checked before the surgical procedure and in the first, second, third and seventh days during the postoperative period to observe pyrogenic reaction due to the synthetic glue or signs of wound infection or meningitis. Rabbit physiological body temperature is quoted in literature as ranging from 38.6°C to 40.1°C.26

In order to evaluate ponderal evolution, the rabbits were weighed before the surgery and in specific days before sacrifice.

During the operation the animals were in a ventral position, the scalp was shaved and then prepared with povidone-iodine solution. A curved fronto-parieto-temporal incision, 1cm parasagittal craniectomy, with 0.5cm dural opening was performed. After closure with 6-0 polypropylene suture, in the first group (A group), 2-octyl-cyanoacrylate was applied. After closure with 6-0 polypropylene suture in the second group (B group), biological glue (fibrin glue) was applied. The glue was placed on the dura mater after closure, taking precaution to avoid dispersion of the glue to the adjacent tissues. In the third (control group), only dural suture was performed. Animals were given a standard diet postoperatively and tap water ad libitum.

We compared commercially available fibrin sealant (Beriplast, Aventis Behring, Marburg, Germany) that has been widely used as a supplementary way for dural closure or CSF leakage treatment, with the 2-octyl-cyanoacrylate tissue adhesive (Dermabond-High Viscosity, Ethicon, Norderstedt): It is in a liquid form that is syrup-like in viscosity, which polymerizes by contact with air, water or blood within minutes. It is marketed in a single-use applicator with 0.5ml of liquid octyl-cyanoacrylate contained in a plastic vial, used as suggested by the manufacturer. Animal studies were approved by institutional review boards.
The rabbits were sacrificed in the seventh, thirtieth and sixtieth days. During Macroscopical postmortem suture examination was done: integrity of the sutures, existence of abscess, wound infection and adhesion formation were recorded.

The heads were carefully removed and placed in 10% formaldehyde. After dural removal and staining with hematoxylin and eosin, histological grading was done, using a 0 to 3 numerical scale (0: no evidence, 1+: occasional evidence, 2+: light scattering, 3+: abundant evidence). Evaluated parameters were inflammatory cell infiltration (white blood count), fibroblast, mononuclear infiltration, foreign body reaction, blood vessel ingrowths (neovascularization) and hemorrhage.

Statistical analysis used the SPSS PC (8.0) program and data were expressed as mean ± SD. For comparing the continuous variables t-Student test and Mann-Whitney U tests were used, when appropriate. ANOVA and Kruskal-Wallis tests were used for several independent samples. A $p$ value of less than 0.05 was accepted as significant.

**RESULTS**

Fifty-four rabbits survived the entire study period. Two animals died of anesthetic overdosage and were excluded from the total of 56 initial rabbits. There were no spontaneous skin dehiscences, CSF leak, abscesses or other infections like meningitis.

The mean body temperature for the days 0 (T0), 1 (T1), 2 (T2), 3 (T3) and 7 (T7) is presented in figure 2. Lower mean temperatures were found in the control group, while A and B groups presented with the highest mean temperatures, however equivalent to normal values (not feverish).

The ANOVA model was used with repeated measures (days) and temperature as a dependent variable: mean temperature did not vary significantly in A group during the days (0, 1, 2, 3 e 7) of the experiment ($p=0.002$), and also for the two other groups. Temperature was not statistically significantly different in the seventh day in the groups ($p=0.210$).

The histopathological variables were studied with descriptive measurements for each day (Table 1): no significant difference did take place daily ($p>0.05$) for all groups. An ANOVA model, with a classification (groups: Control, A and B) for each variable was used: for each day of the experiment, the differences of the dural degree of histological alteration of each experimental unit per group was evaluated. We noticed that vascularization presented significant results in 7th and 60th days: a small neovascularization took place in group A and the biggest one in the control group (Tukey test). There was no significant difference between A and B groups. In the 60th day, a mild neovascularization took place in group B and greater in group A, but the difference between the A and B groups was inferior to the unit used to evaluate cellularity.

**Fig. 1**: Initial and final mean weight for group.

Mean weight of each rabbit of the synthetic glue group increased, demonstrating that this glue didn’t interfere in the metabolism of these animals, what reflects a toxicological advantage comparing to other synthetic products. Except in a control group, we observed a small increase of weight (0.07%) in six rabbits. In B and A groups a reasonable increase in weight could be respectively noticed, B = 4.41% and A = 8.04% (Fig 1). Control group mean weight almost did not vary but there was an increase in groups B and A, without statistical significance ($p=0.064$). A group presented a significant increase of mean weight (t-Student test), when comparing the initial and final mean weight ($p=0.004$).
Table 1. Comparison (days 7, 30 and 60) of the Control, B and A groups of the histopathologic variables with the Kruskal-Wallis test and descriptive measurements.

<table>
<thead>
<tr>
<th>Histopathology</th>
<th>Day</th>
<th>Statist.</th>
<th>Value-P</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroblast</td>
<td>7</td>
<td>3.36</td>
<td>0.186</td>
<td>2.17</td>
<td>0.98</td>
<td>1.50</td>
<td>0.84</td>
<td>1.17</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.00</td>
<td>1.000</td>
<td>0.00</td>
<td>0.0</td>
<td>1.00</td>
<td>0.00</td>
<td>1.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>2.00</td>
<td>0.368</td>
<td>0.00</td>
<td>0.0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.17</td>
<td>0.41</td>
</tr>
<tr>
<td>Acute Inflammatory Reaction</td>
<td>7</td>
<td>3.79</td>
<td>0.150</td>
<td>0.00</td>
<td>0.0</td>
<td>0.33</td>
<td>0.52</td>
<td>0.67</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.00</td>
<td>1.000</td>
<td>0.00</td>
<td>0.0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.00</td>
<td>1.000</td>
<td>0.00</td>
<td>0.0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Mononuclear Infiltrate</td>
<td>7</td>
<td>1.70</td>
<td>0.427</td>
<td>1.00</td>
<td>1.1</td>
<td>0.33</td>
<td>0.86</td>
<td>0.50</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3.662</td>
<td>0.160</td>
<td>0.33</td>
<td>0.52</td>
<td>0.00</td>
<td>0.00</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.00</td>
<td>1.000</td>
<td>0.00</td>
<td>0.0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Foreign Body</td>
<td>7</td>
<td>0.00</td>
<td>1.000</td>
<td>0.00</td>
<td>0.0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.00</td>
<td>1.000</td>
<td>0.00</td>
<td>0.0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.00</td>
<td>1.000</td>
<td>0.00</td>
<td>0.0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Vascularization</td>
<td>7</td>
<td>5.69</td>
<td>0.058</td>
<td>2.17</td>
<td>0.98</td>
<td>1.17</td>
<td>0.41</td>
<td>1.00</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>4.70</td>
<td>0.096</td>
<td>0.50</td>
<td>0.55</td>
<td>0.33</td>
<td>0.52</td>
<td>1.17</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>5.74</td>
<td>0.057</td>
<td>0.50</td>
<td>0.55</td>
<td>0.33</td>
<td>0.52</td>
<td>1.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>7</td>
<td>0.81</td>
<td>0.668</td>
<td>1.17</td>
<td>0.75</td>
<td>1.33</td>
<td>1.03</td>
<td>1.00</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.06</td>
<td>0.588</td>
<td>0.00</td>
<td>0.0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.17</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.00</td>
<td>1.000</td>
<td>0.00</td>
<td>0.0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Fig. 3: Box Plot diagram for the histological variables fibroblast, acute inflammatory reaction (AIR) and vascularization.

The fibroblast concentration progressively decreases comparing the Control group (bigger values) with B and A groups (Fig. 3). In the glue groups, the values of B were similar to the behavior of the values of group A. The AIR takes place in the presence of the biological glue (B) or synthetic glue (A). Blood vessel ingrowth was less in A and B groups compared with control group.

Wound healing process, as assessed by inflammatory cell infiltration, neovascularization and fibroblast activity, did not differ significantly between the three groups in the seventh, thirtieth and sixtieth days ($p>0.05$) (Fig. 4). There was no difference between the groups regarding mononuclear cell infiltration ($p>0.05$), foreign body ($p>0.05$), and hemorrhage ($p>0.05$).

Fig. 4: Photomicrographs of histological specimens obtained in groups A and B on seventh day. Hematoxylin and Eosin, original magnification X 400. (A) Fibroblast proliferation in group A. (B) Fibroblast proliferation in group B. (C) Acute inflammatory reaction (AIR) in group A. (D) AIR in group B.

Discussion

Some neurosurgical procedures have high morbidity and mortality rates due to CSF fistula development. Dural laceration is very common after head injury, dural tumors like meningiomas and cranial surgery in old patients, where the dura is attached to the bone, particularly when dural defects are surrounded by friable dura or in relatively inaccessible areas, such as the axilla of the nerve root sheath or in neural foramen as a complication of spinal surgery when a dural tear occurs. The sequel of dural laceration includes dural may include cutaneous fistulas, arachnoiditis, meningitis and neurological dysfunction in cranial surgery and pseudomeningocele in spinal surgery.

After conservative treatment of a persistent CSF leakage through a surgical wound, surgical intervention may be required,
with closure of the leak. As an alternative, tissue sealants and adhesives have been used to stop CSF leaks\textsuperscript{10,12,23,28}, most of these are adjuncts for a good dural closure.

We used a rabbit model to test 3 different dural closure techniques to determine if anyone would be significantly superior to the others, by evaluating histotoxicity through the inflammatory response and foreign body giant-cell reaction at the site of application, with minimal harm to dural and adjacent tissue.

Cyanoacrylates were first reported in 1949 and were used as tissue adhesives by Coover et al\textsuperscript{3}. Cyanoacrylate tissue adhesive has therefore expanded to clinicians’ options for wound closure\textsuperscript{10}. These synthetic adhesives have been used to achieve repair of blood vessels, solid organ injuries, dural defects, carotid cavernous sinus fistulas and arteriovenous malformations\textsuperscript{4}. Cyanoacrylate also has been used to treat intracranial aneurysms for a long time\textsuperscript{13}, in otolaryngology\textsuperscript{21}, in facial plastic surgery, and has been an alternative to suturing a variety of types of lacerations and scalp wounds\textsuperscript{10}.

These cyanoacrylate monomers polymerize in the presence of the hydroxyl ions in water and blood, and are able to bind the edges of the epithelial layer in a wound together\textsuperscript{4}.

Reports of cytotoxicity have been hampered by the acceptance of cyanoacrylates polymers\textsuperscript{27}.

Longer alkyl-chain cyanoacrylates, like the n-butyl-cyanoacrylate and the 2-octyl cyanoacrylate, are less toxic and maintain a stronger bond than shorter chain cyanoacrylates (methyl, ethyl), that are more reactive and have a greater toxicity. The n-butyl-cyanoacrylates at the site of application produce a mild inflammatory response and foreign body giant-cell reaction and are biodegradable. The 2-octyl-cyanoacrylates are even more stable, have greater flexibility and maintain a stronger bond: they also degrade much more slowly than butyl-cyanoacrylates and are considered to be nontoxic\textsuperscript{14,27}. There are no reports of systemic toxicity associated with the use of topical octyl-cyanoacrylates\textsuperscript{10}.

Fibrin sealant, the biological glue, is another type of adhesive that has been used in conjunction with dural and other sutures to stop or control bleeding, or to provide air and fluid tightness in many surgical situations\textsuperscript{22}. Its use has led to successful sealing of CSF leaks\textsuperscript{12,20,23}. But because fibrin glue components (fibrinogen and thrombin) are extracted from pooled human plasma, their use may permit transmission of infectious diseases or induce an anaphylactic reaction\textsuperscript{10,24}.

Agrawal et al., 1998, in an experimental study in 20 rats, studying histopathological changes following the use of biological and synthetic glue for dural grafts, noticed an intense inflammatory reaction observed with both glues, which was found to persist till the eleventh week\textsuperscript{4}.

Ozisik et al., 2006, used a rat model to test 4 different dural closure techniques. They used methyl-metacrylate, n-butyl-cyanoacrylate, fibrin glue and CO\textsubscript{2} laser and concluded that fibrin glue was the safest material with a CSF leakage risk that was higher but acceptable than n-butyl-cyanoacrylate (25% vs 12.5%)\textsuperscript{16}. Methyl-metacrylate and CO\textsubscript{2} laser techniques were inadequate for stopping dural leakage\textsuperscript{16}.

Cain et al., 1988, investigated the relative strength of dural repair using standard suture techniques, suture supplemented with tissue adhesive, and tissue adhesive alone. They observed that defects in cadaveric dura, repaired only with suture leaked at pressurization within the physiological range, while those supplemented with tissue adhesives failed at higher pressurization levels; in other words, they have shown that both simple interrupted and running locked suture techniques used in vitro on human dura samples failed to maintain a watertight seal at pressurization within the normal physiological range. When identical suture techniques were reinforced with fibrin glue or cyanoacrylate polymer, the in-vitro strengths were improved sevenfold and 22-fold, respectively. In the same paper, in-vitro testing, accomplished in New Zealand rabbits, reached this conclusion, and differently to our study, histological sections obtained from dura treated with fibrin adhesive sealant demonstrated minimal inflammatory response, while those sections obtained at the site of dural repair augmented with cyanoacrylate polymer (ethyl-2-cyanoacrylate) featured significant inflammatory responses, including dural thinning, gliosis and cortical necrosis\textsuperscript{5}. This was done to the use of a liquid form of cyanoacrylate, different from that used by us, a high viscosity form, decreasing the risk of leakage through the dura. They also noticed differences in the failure pressures between the human cadaveric dura and the in-vitro rabbit dura samples treated with identical methods. The authors attribute these differences to the relative dural thickness of the two different species\textsuperscript{5}.

Toriumi et al., 1991, studied butyl-2-cyanoacrylate applied between bone graft and cartilage in one rabbit’s ear and adjacent to well-vascularized soft tissue with no graft in the opposite ear. Histological analysis revealed minimal if any inflammation when small amounts of glue were used in the nonvascular region between bone graft and cartilage. However, subcutaneous implantation contacting well-vascularized soft tissue resulted in increased acute inflammation and prolonged foreign body giant-cell response\textsuperscript{27}. In our study, 2-octyl-cyanoacrylate elicited minimal histotoxicity when used on the sutured rabbit dura, because dural tissue has a fibrous structure and low vascularization compared to the soft tissue. The studies that used more toxic derivatives of the cyanoacrylate adhesive (like shorter alkyl-chain molecules, methyl and ethyl) have shown histotoxicity and an intense inflammatory
reaction\(^1,5,16\). This reaction is due to the cyanide residue and to the heat released when the monomer polymerizes\(^1\). This newer derivative, 2-octyl-cyanoacrylate, with longer side chains, polymerizes slower, so releasing heat to the tissues more slowly, and theoretically nontoxic\(^2\). However, as shown in this study, it is almost free of the inflammatory adverse properties of the earlier glues.

Dural defects and CSF leaks are common neurosurgical problems and the search for a suitable sealant continues. The effects of 2-octyl-cyanoacrylate over the meninges have not been previously studied and we conclude that this synthetic glue is advantageous in experimental dural healing, with similar behavior with the standard used fibrin glue. Although it may be advised for superficial use, it seems to be appropriate as an adjunct for dural closure.

### REFERENCES


DISCLOSURE STATEMENT

The authors have no conflicts of interest to declare that could inappropriately influence the here presented work.

CORRESPONDING AUTHOR

Maurus, M.A. Holanda
Endereço: Rua Santos Coelho Neto, 200/802, Manaíra, 58038-450, João Pessoa, PB, Brasil
Telefone: +55 83 9302 8858
Fax: +55 83 3222 7167
E-mail: maurusholanda@hotmail.com