ABSTRACT

Objective: To study the feasibility of heterologous aponeurosis to close the abdominal wall of rabbits emphasizing the rejection process. Methods: This project was approved by the Animals Care Committee of the Faculdade de Ciências Médicas da Santa Casa de São Paulo, and it was carried out at the Experimental Surgery and Technical Unit. Four red female rabbits exchanged abdominal wall aponeurosis with other four white male animals. Two rabbits just had it removed and replaced to be the control group for the healing process. Animals were evaluated once a day, and after 7 days they were sacrificed. Immunohistochemical analysis with CD20 and CD79 was done. Results: The animals did not have cellulitis, abscess, hematoma, wound dehiscence or herniation. The graft site showed intense hyperemia. The histological analysis showed an inflammatory process with maturing myofibroblasts and collagen ranging from incipient to moderate. The number of vessels was reduced and the inflammatory cells were most plasma cells and macrophages. There were no morphological signs of acute rejection with aggressive vascular endothelial damage. The adjacent muscle showed signs of degeneration with inflammatory centralization of nuclei and cytoplasmic condensation. The immunohistochemical analysis (CD20 and CD79) showed that the inflammatory process was not mediated by lymphocytes. Mann-Whitney nonparametric test showed that the hypothesis of equality (p>0.05) should not be discarded. Conclusion: There were no postoperative complications (fistulas, dehiscence etc.) and the histological analysis showed nonspecific inflammatory process. The immunohistochemical analysis showed that the inflammatory process was not due to a possible rejection.

Keywords: Graft rejection; Antigens, CD20; Transplantation; Abdominal wall/surgery; Rabbits

RESUMO

Objetivo: Estudar a viabilidade de aponeurose heteróloga para fechar parede abdominal de coelhos, com ênfase no processo de rejeição. Métodos: Este projeto foi aprovado pelo Comitê de Cuidados Animais da Faculdade de Ciências Médicas da Santa Casa de São Paulo e realizado na Unidade Técnica e Cirurgia Experimental. Quatro coelhas vermelhas trocaram aponeurose da parede abdominal com outros quatro animais machos brancos. Em dois coelhos, foi retirada e substituída a aponeurose como controle do processo cicatricial. Eles foram avaliados 1 vez por dia e sacrificados após 7 dias. Foi realizada a imunoistoquímica com CD20 e CD79. Resultados: Os animais não tiveram celulite, abscesso, hematoma, deiscência da ferida ou hérnia. O local do enxerto mostrou hiperemia intensa. A análise histológica mostrou um processo inflamatório, com a presença de miofibroblastos em amadurecimento e colágeno, que variou de incipiente a moderado. O número de vasos estava reduzido e as células inflamatórias foram, em sua maioria, células plasmáticas e macrofagos. Não havia sinais morfológicos da rejeição aguda com a agressão do endotélio vascular. O músculo adjacente mostrou sinais de degeneração, com reação inflamatória dos núcleos e condensação do citoplasma. A análise imunoistoquímica (CD20 e CD79) mostrou que o processo inflamatório não foi mediado por linfócitos. O teste não paramétrico de Mann-Whitney mostrou que não se pode rejeitar a hipótese de igualdade (p>0,05). Conclusão: Não houve complicações pós-operatórias (fístulas, deiscência etc.) e a análise histológica revelou processo inflamatório inespecífico. A análise imunoistoquímica mostrou que o processo inflamatório não foi em razão de uma possível rejeição.

Descritores: Rejeição de enxerto; Antígenos CD20; Transplante; Parede abdominal/cirurgia; Coelhos
INTRODUCTION
The loss of integrity of the abdominal wall may occur due to trauma, infection, herniation or surgical resection\(^1\). To deal with these wall defects the aim is to achieve the functional and aesthetic recovery of organs and affected tissues\(^2,3\).

In some cases of intestinal and multivisceral transplantation, for example, the abdominal wall closure is a major technical challenge. In approximately 20% of cases there is not enough tissue to perform a primary wall closure\(^4\). The failure of primary repair after a transplant leaves an exposed wound that will require new surgical approaches and a long rehabilitation period.

Complications are potentially lethal and include intra-abdominal infection, injury to unprotected organs, bleeding and fistulas\(^5\). There are several surgical options for closing the abdominal wall: primary closure, tissue expansion, use of synthetic prostheses, components separation, and depend on flap localization and distance\(^6\).

In cases in which the muscle-aponeurotic coverage is inadequate, the use of synthetic material is the main applied technique\(^1\). However, beyond the implications of costs, its use is contraindicated in some conditions such as concomitant gastrointestinal fistulae, enterocutaneous fistula, necrotizing fasciitis, contaminated wounds and immunosuppression, as well as foreign body rejection and infection\(^7\).

The advent of absorbable biological implants proved advantageous regarding the ability to be assimilated by the body and because they do not need to be removed in case of infection or wound dehiscence\(^8\). Human fascia lata, swine extracellular collagen matrix, human dura mater and bovine pericardium are the most widely used types of implants\(^9\).

Fascia lata graft has been used in several medical specialties. In ophthalmology, it is used as orbital floor reconstruction or as a suspensor in frontal lid cases of ptosis. In neurosurgery, it is used to reconstruct the dura mater during tumor resection. In orthopedics, it is commonly used for ligament reconstruction and, in urology, it is used to treat female urinary incontinence as pubovaginal suspensor\(^10,12\).

Peláez Mata et al. presented aesthetics and functional satisfactory abdominal wall closing using fascia lata from cadavers to reconstruct abdominal wall defects in children\(^13\). Disa et al. indicated a rate of 3% of recurrent hernias in 32 patients undergoing abdominal wall reconstruction with autologous fascia lata in a 9-year postoperative follow-up\(^14\).

Fascia lata graft to repair large abdominal wall defects is resistant and supports intra-abdominal pressures, properly integrating the receiving area\(^15,16\). Integrated grafts keep the collagen fibers architecture and orientation, and they are easily embedded into the fibrocolagenous tissue without causing foreign body reaction\(^17\). The anchorage of the graft edges on the muscle-aponeurotic defect proved to be better than any synthetic graft\(^18\).

Since the early 1990s there are fascia lata banks that allow the safety use of such grafts in orthopedics and ophthalmology\(^19\). Risk of rejection and the transmission of diseases, according to the Food and Drug Administration (FDA), are considered minimal\(^7,20\).

However, the withdrawal of human fascia lata involves surgery and results in mutilation of patients, besides this implant limited stock. The size of the graft, even considering a bilateral withdrawal, is also restricted, and this is another problem in case of large defects\(^11,21\). In addition, there may be hematomas, seromas and wound dehiscence at the donor site\(^14\).

There are no references in the literature on the use of the aponeurosis in heterologous abdominal wall closure. It is proposed here an experimental study using an aponeurosis of rectus abdominis in no syngeneic rabbits.

OBJECTIVE
To study the feasibility of using heterologous aponeurosis rectus abdominis for abdominal wall closure based on the macroscopic and histological analysis of the graft emphasizing the rejection process.

METHODS
This project was approved by the Animals Care Committee of Faculdade de Ciências Médicas da Santa Casa de São Paulo (SCMSP), and it was carried out at the Experimental Surgery and Technical Unit (UTECE) of this institution. Four male New Zealand white rabbits and four female New Zealand red rabbits, weighing between 3 and 4kg, were divided into two study groups, as described below.

In Group A (n=4), males have undergone partial resection of the anterior aponeurosis of the rectus abdominis and received aponeurosis grafts of equal size as animals of Group B. In Group B (n=4), female animals were submitted to partial resection of the anterior aponeurosis of the rectus abdominis and...
received aponeurosis grafts of equal size as animals in Group A. One animal from Group A was the recipient and donor for the same Group B animal and vice-versa (Figure 1).

A white male and a red female weighing between 3 and 4kg formed the control group (Group C). In this group (n=2), the aponeurosis of the animals were sectioned and reincorporated to the same segment of the anterior aponeurosis of the rectus sheath (Figure 1).

**Preparing the graft**
The resected segment of the aponeurosis was rinsed in a 0.9% saline solution at 4°C, than the segments of aponeurosis were sutured to the edges of the aponeurotic defect using a 4-0 nylon stitch (Figure 2).

**Post-operative**
The rabbits were housed for 3 weeks at the UTECE. Right after the postoperative period they were offered *ad libitum* access to food and water. Every day, once a day, the wound was examined for the presence of cellulitis, abscess, hematoma, dehiscence of the suture and evagination. When necessary, if the animal showed discomfort in the postoperative period, it immediately received analgesia with morphine 2 to 5mg/kg subcutaneous every 4 hours. The animals were sacrificed 7 days after the surgery by an intravenous potassium chloride (19.1%) 10mL injection. After performing an extensive infra-umbilical transverse incision, the graft was removed with a 1-cm margin.

**Histological evaluation of the graft**
Histological preparation was carried out by the Pathology Service of SCMSP and the analysis was performed at Cardoso de Almeida Pathology Service. Samples of 0.5cm by 0.5cm consisting of equal aponeurosis segment lengths from donor and recipient were preserved in a formaldehyde solution and fixed in paraffin blocks. Histological changes were evaluated according to the semi-quantitative system described by Hooker et al. (22). The analysis of the rejection process was performed by immunohistochemistry reagents for CD20 and CD79.

**RESULTS**
The animals did not have cellulitis, abscess, hematoma, wound dehiscence or herniation. The graft site showed intense hyperemia when animals were sacrifice 7 days after the surgery. The histological analysis showed an inflammatory process, with maturing myofibroblasts and collagen, ranging from incipient to moderate. The number of vessels was reduced and the inflammatory cells were mostly plasma cells and macrophages. There were no morphological signs of acute rejection with aggressive vascular endothelia. The adjacent muscle showed signs of degeneration with an inflammatory centralization of nuclei and cytoplasm condensation (Figure 3). The immunohistochemical analysis (CD20 and CD79) showed that inflammatory process was not mediated by lymphocytes. The nonparametric Mann-Whitney test showed that the hypothesis of equality (p>0.05) could not be rejected.
complete integration with revascularization and with maintenance of the graft structural conformation, an adequate support for intra-abdominal pressure, lata aponeurosis in abdominal wall closure, it provides its application. Regarding the use of autologous fascia showed a line of research with the use of heterologous plasma cells and macrophages

DISCUSSION

The current use of synthetic materials to reconstruct the integrity of abdominal wall is related to high rates of infection and rejection, and its use is contraindicated in some situations. The cost is also a limiting factor for its application. Regarding the use of autologous fascia lata aponeurosis in abdominal wall closure, it provides an adequate support for intra-abdominal pressure, with maintenance of the graft structural conformation, complete integration with revascularization and decreased risk of graft reaction. The problem is its limited stock besides the mutilation of another region of the patient's body.

There are no experimental studies in the literature evaluating the use of heterologous aponeurosis of the rectus abdominis muscle for abdominal wall closure to establish comparisons. The animals of the study did not present postoperative complications (fistulas, dehiscence etc.) and the histological analysis showed a nonspecific inflammatory process. The immunohistochemical analysis also showed that the inflammatory process was not mediated by lymphocytes.

Despite being preliminary, these results showed that the inflammatory process was not due to a possible rejection.

CONCLUSION

The use of heterologous aponeurosis in abdominal wall closure was feasible and no signs of rejection were shown on the immunohistochemical analysis. This study also showed a line of research with the use of heterologous aponeurosis in abdominal wall closure.

REFERENCES


