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Experimental model of anal fistula in rats

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ABSTRACT

Introduction: the management of anal fistula remains debatable. The lack of a standard treatment free of complications stimulates the development of new options.

Objective: to develop an experimental model of anal fistula in rats.

Methods: to surgically create an anal fistula in 10 rats with Seton introduced through the anal sphincter musculature. The animals were euthanized for histological fistula tract assessment. **Results:** all ten specimens histologically assessed had a lumen and surrounding granulation tissue. There was complete epithelialization of the tract in two samples, halfway epithelialization in one sample and epithelialization of only the outer portion in six samples. Epithelialization was not evident in one tract.

Conclusion: anal fistulas in rats were histologically proved.

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Modelo experimental de fístula anal em ratos

RESUMO

Introdução: o manejo da fístula anal ainda permanece um debate. A ausência de um tratamento ideal isento de complicações estimula o desenvolvimento de novas modalidades terapêuticas.

Objetivo: desenvolver um modelo experimental de fístula anal em ratos.

Metodologia: criação de fístula anal cirúrgica em 10 ratos por meio de passagem de fio de aço através da musculatura do esfíncter anal. Os animais foram submetidos a eutanásia para comprovação histológica do trajeto fistuloso.

Resultados: todos os segmentos analisados histologicamente apresentaram lúmen e tecido de granulação. Houve epiteliação completa do trajeto em dois espécimes, epiteliação até a metade do trajeto em um, e epiteliação somente da porção externa em seis. Um trajeto não apresentou área de epiteliação.

Conclusão: o desenvolvimento de fístula anal em ratos foi comprovado histologicamente.

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Palavras-chave:

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Introduction

The management of anal fistula remains a debatable issue as there is no single technique suitable for the treatment of all anal fistulas.

Complex fistulae are characterized by a greater risk of continence alterations and therefore, there is great interest in assessing new therapeutic methods for these cases. The development of experimental models of anal fistula arises as an aid for these new researches.

Objective

The study aimed to develop an experimental model of anal fistula in rats to study new treatment strategies.

Material and methods

The project was approved by the Ethics Committee on Animal Use (CEUA) of Universidade Federal de Mato Grosso do Sul (UFMS) for the use of animals in research. A total of 10 albino Wistar adult male rats (*Rattus norvegicus*) were used, weighing approximately 300 g. The animals were kept in the experimentation sector of the Central Animal Laboratory of UFMS, according to all required ethical standards.

Anesthesia was performed by intraperitoneal administration of ketamine and xylazine association in the same syringe. The solution consisted of 1.0 mL of ketamine and 10%, 1.0 mL xylazine 2%. For each 100 g of body weight, 0.1 mL of the anesthetic solution was infused.

Fistula creation technique

A total of 10 animals were used. The animals were placed in the supine position, with the four limbs in abduction. The entire procedure was performed under sterile conditions, complying with all the care of antisepsis and asepsis of the operated region.

A threaded steel wire number 5 (Aciflex®) was passed through the anal sphincter musculature approximately 1 cm lateral to the anal margin to the left (Fig. 1). After perforation of the skin by the needle, the steel wire was cut and tied loosely through rotating movements around the anal sphincter muscle (Fig. 2).

The animals were maintained in individual cages during the study period with food and water *ad libitum*. They were checked daily and water and food were changed weekly.

After 30 days the animals were sacrificed for histological demonstration of fistula. The drug used for euthanasia was sodium thiopental by intracardiac administration after sedation with ketamine and xylazine.

Histological evaluation of the fistula tract

After euthanasia, a circumferential incision around the anus was performed using a cold scalpel, including the perineum and a margin lateral to the steel wire tract. Dissection was

performed with removal of the piece *en bloc*, extending to the rectum (Fig. 3).

The resected material was sectioned and submitted to staining methods for histological analysis. The slides were analyzed for evidence of fistula tract by evaluating each side of the fistula lumen (internal and external orifice), presence of lumen, granulation tissue and area of epithelialization.

Results

On the thirtieth day, all animals had the steel wire properly tied around the anal sphincter, with no signs of corrosion or destruction of the Seton by the animal.

Histologically, all 10 specimens analyzed showed a lumen surrounded by granulation tissue (Fig. 4).

Concerning the epithelialization area, two specimens showed complete epithelialization of the tract (Fig. 5); one specimen showed half-way epithelialization and six specimens showed epithelialization of the outer portion. One fistula tract showed no epithelialization area, but only granulation tissue.

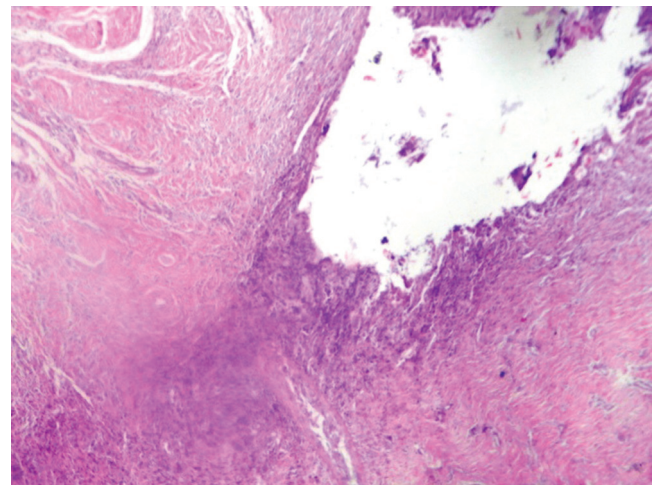


Fig. 1 – Passage of steel wire through the anal sphincter muscles.



Fig. 2 – Experimental model ready with steel wire loosely tied around the anal sphincter muscles.

Discussion

Cryptoglandular infection is an etiologic factor of idiopathic anal fistula that is more acceptable in patients with no

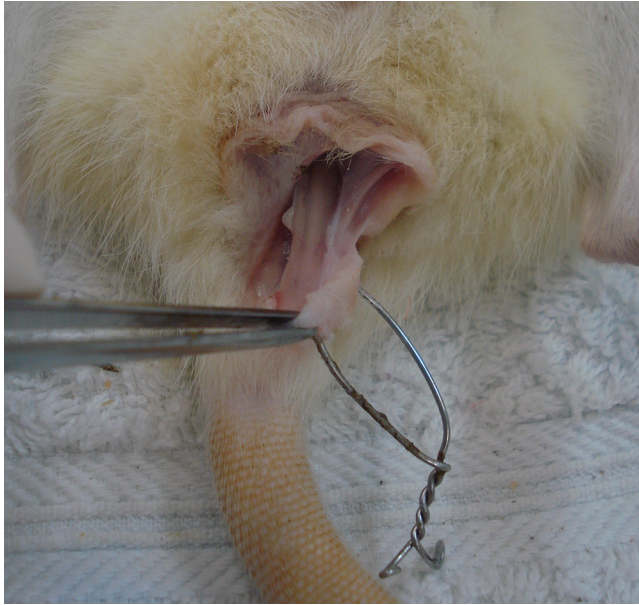


Fig. 3 – Dissection around the anus with lateral margin to the metal wire tract, extending to the rectum.

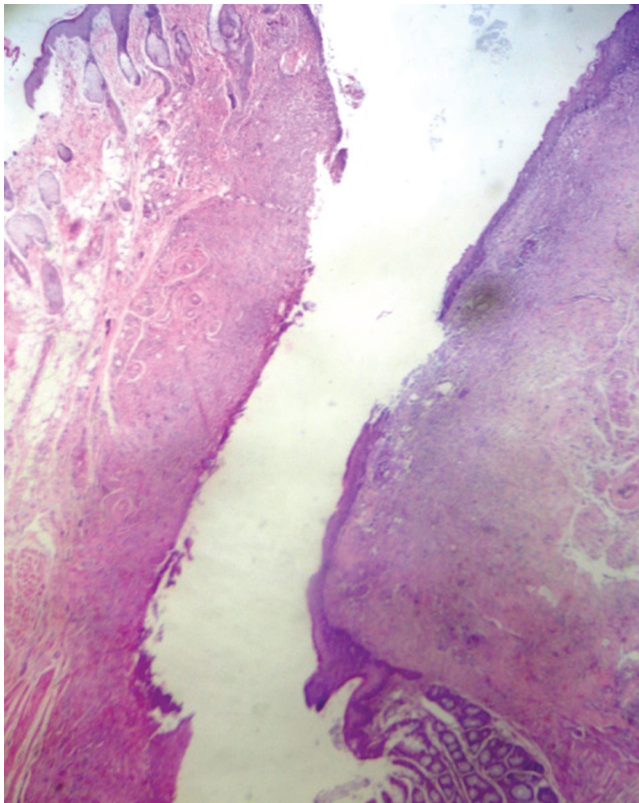


Fig. 4 – Presence of lumen and granulomatous reaction with giant cells. Presence of food debris; non-epithelialized tract.



Fig. 5 – Panoramic photo showing completely epithelialized fistula.

comorbidity. The anal glands penetrate the anal sphincter at varying degrees and locations. Once obstructed, there is infection, abscess formation and subsequently, the fistula onset.¹

The anal fistula is a common problem, with an incidence of 9 per 100,000 individuals² and its treatment can bring some complications such as pain and anal incontinence.³

Fistulas can be classified as simple or complex. Simple anal fistulas can be treated by fistulotomy with success rates between 92% and 97%, but higher rates of recurrence and postoperative continence alterations resulting from this procedure have been associated with complex fistulas.⁴

Due to the difficulty of performing complete histological study to evaluate treatments, this study explored the possibility of developing an experimental model for this purpose. Taking into account the need for new studies to find a more effective treatment for anal fistulas, it is essential to have an experimental model that is equivalent to fistulas in humans and that is low cost and easy to perform.

The use of rats in the experiment was justified by the fact that the surgical procedure in rats was easier, due to the small size as well as availability of the animals, and the fact that there was no description in the literature of the use of these animals as experimental models, with reports found only in dogs⁵ and pigs.⁶ Rats have the structure of the internal and external sphincters similar to humans. It is known that, unlike human mucous anal glands, rats have the simplest type of sebaceous glands involving the anal canal and located mainly in the submucosal region. It is believed that dogs have anal glands in the internal sphincter similar to anal glands in humans, but in a study performed in these animals, it was not possible to develop a fistula tract by ligation of the drainage orifice of the gland, as it was carried out in sweat glands.⁵

The experimental model of artificial anal fistula most accepted in the world was developed in pigs.⁶ Although the model has been successful, as demonstrated by MRI, presence of lumen and granulation tissue at histological analysis and the fact that it has been used for the study of new techniques, we believe that a similar model in rats would be easier to reproduce, with further contribution to advances in the study of techniques for the treatment of fistulas. In the present work, lumen and granulation tissue

were observed in all specimens, as well as the presence of some degree of epithelialization in 90% of fistulas. The work of Buchanan *et al.*⁶ showed no epithelialization in any of the fistula tracts produced, despite the Seton permanence for 26 days.

The placement of steel wire as inducer of the inflammatory process for the development of fistula was chosen because it is a material difficult to be destroyed by the animals. The material was apparently well tolerated and in the end of the thirtieth day there was no wire destruction or change in wire position or quality.

At the histological evaluation of this study, the outer portion was the most frequent location of epithelialization, corroborating the hypothesis that epithelialization of perianal fistulas starts on the side of the external orifice, serving as a defense mechanism to prevent local and systemic infections caused by microorganisms from the stool that passes through the fistula tract.⁷ Full epithelialization of the tract is a more difficult event to be observed.

Epithelial growth in perianal fistulas is a late event; however, the time between the fistula appearance or presence of Seton and epithelialization onset is not estimated. It is not known why epithelialization does not occur in all fistulas during the same evolution period, but it has been suggested that the perpetuation of inflammation prevents the migration and arrangement of myofibroblast cells that are crucial in the tissue recovery process.⁷

It is believed that the epithelialization of the fistula tract may contribute to treatment failure and fistula persistence, but the work of Mitalas *et al.*⁷ showed no difference in the results achieved in fistulas with or without epithelialization.

Conclusion

The development of anal fistula in rats was confirmed histologically through the placement of a metallic Seton for thirty days.

Conflicts of interest

The authors declare no conflicts of interest.

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