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Differentiation of myofibroblasts in wounds after topical use of metronidazole: an experimental study.

Diferenciação de miofibroblastos em feridas após uso tópico do metronidazol: estudo experimental.

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A B S T R A C T

Objective: to assess the effects of topical administration of metronidazole on fibroblast differentiation and on wound contraction during experimental secondary intention wound healing in rats. **Methods:** we submitted 108 rats to a circular wound on the back, 2cm in diameter, and divided them into six groups: control group, with application of saline solution on the wound and five experimental groups, divided according to the concentration of metronidazole solution used (4%, 6%, 8%, 10% and 12%). We changed the dressings daily throughout the trial period, which comprised three stages of analysis: three, seven and 14 days. We evaluated wound contraction by digital planimetry, and identified myofibroblasts and protomyofibroblasts using CD34 and α-SMA immunohistochemistry techniques. **Results:** wound contraction was not different between the experimental and the control groups. Protomyofibroblasts were significantly more numerous at seven days (p=0.022) in the 4%, 6% and 8% metronidazole groups. After 14 days, in the same groups, myofibroblasts predominated significantly (p=0.01). **Conclusion:** the topical administration of metronidazole solution in skin wounds healing by secondary intention was able to improve the differentiation of fibroblasts. The contraction phase of wound healing remained unchanged, without significant reduction of the contraction evaluated by digital planimetry. These results can be used in favor of the wound healing process.

Keywords: Wound Healing. Metronidazole. Administration, Topical. Fibroblasts. Myofibroblasts.

INTRODUCTION

The wound healing process is the subject of research for centuries, but in the last four decades, there has been intensified work on fibroplasia with interest in functional or cosmetic rehabilitation[1-3].

After skin trauma, accidental or surgical, factors related to tissue repair are activated, evolving in stages: coagulation, inflammation, proliferation, wound contraction and remodeling. But these phases may be modified in various situations, such as when there is infection, which perpetuates the inflammatory process and causes the chronification of a skin lesion, such leg ulcers or, conversely, when using topical creams and special dressings that accelerate the process of fibroplasia, in aesthetic procedures[4].

The phenomenon of wound contraction, that is, the relationship between wound size and reduction rate during healing, completed 100 years of its description[5]. However, the main cell responsible for this phenomenon, the myofibroblast, was only described after decades. In 1971, Gabbiani[6] observed fibroblasts present in granulation tissue that had their cytoplasm similar to those found in smooth muscle cells, thus describing the myofibroblast. In 1990, an experimental study with open wounds in rat skin demonstrated that the myofibroblasts derived from the local fibroblasts, after developing muscle fibrils bands, called alpha smooth muscle actin (α-SMA), a characteristic that identifies the myofibroblast by immunohistochemistry[7].

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Recent papers[8,9] stated that the knowledge of the myofibroblasts' activating and blocking factors is a possible key for the treatment and control of neoplasms of stromal origin, such as sarcomas.

Topical metronidazole has been used to odor control in malignant fungating wounds. These are elevated over the skin in a mushroom-like fashion, with large folds, similar to villi and crypts, which provide marked proliferation of anaerobic bacteria, resulting in foul odor lesions[10]. This study aimed to evaluate, in an experimental model of wound healing by secondary intention, the influence of topical metronidazole solution at different concentrations on the amount of protomyofibroblasts and myofibroblasts and its potential role in the contraction phase of wound healing.

METHODS

The project was approved by the Committee on Ethics in the Use of Animals of the Pontifical Catholic University of Paraná - CEUA PUCPR, under protocol # 655.

The experimental study used 108 rats *(Rattus norvegicus, Rodentia mammalia)* of the *Wistar* strain, aged 110 days and average weight between 300g and 315g. They were kept in appropriate individual cages for the species under controlled environmental conditions (12 hours light/dark cycle), with free access to water and standard chow for the species. We estimated the sample size through studies conducted in the researched literature[4,11], and the project followed the guidelines of Law 11794/2008, regulated by decree number 6899, and the recommendations of the Brazilian College of Animal Experimentation.

We randomly divided the animals into six groups. With the exception of the animals in the control group (CG), all the other experimental groups (EG), I to V, underwent dressings with the use of metronidazole in topical solution to 4%, 6%, 8%, 10% and 12%, once a day. Each group was subdivided into three subgroups of six animals for evaluation at three, seven and fourteen days after the skin lesion (Table 1).

Table 1. *Division of animals by groups and evaluation days.*

Groups	Number of rats	Evaluation days
CG*	n-6	3 days
	n-6	7 days
	n-6	14 days
EG** I (4%)	n-6	3 days
	n-6	7 days
	n-6	14 days
EG** II (6%)	n-6	3 days
	n-6	7 days
	n-6	14 days
EG** III (8%)	n-6	3 days
	n-6	7 days
	n-6	14 days
EG** IV (10%)	n-6	3 days
	n-6	7 days
	n-6	14 days
EG** V (12%)	n-6	3 days
	n-6	7 days
	n-6	14 days

** CG: control group; ** EG: experimental group.*

We anesthetized the animals with ketamine 80mg/kg and xylazine 8mg/kg and performed tricotomy in the dorsal region of approximately 24cm^2. After antisepsis with polyvinylpyrrolidone-iodine (PVPI) and delimitation of the operative area with a fenestrated sterilized field, we resected a circular skin segment with a metal punch with 2cm in diameter to expose the dorsal muscular fascia.

All animals had their wounds cleaned with saline solution. In the control group (CG), we applied dressing with dry gauzes. In the five experimental groups (EG), we applied dressings with gauzes embedded in metronidazole solution (benzoylmetronidazole), with EG I at a concentration of 40mg/ml (4%), EG II 60mg/ml (6%), EG III 80mg/ml (8%), EG IV 100mg/ml (10%), and EG V 120mg/ml (12%). After the procedure, the animals received intravenous dipyrone at a dose of 10mg/kg for analgesia.

The wound remained occluded until anesthesia recovery. After this period, the animals were kept in individual cages, which were placed on shelves at equal distance from the light source, receiving water and balanced feed *ad libitum*. On the remaining days, always in the morning, we cleaned the wounds with saline; the CG animals then received 0.3ml of the same saline solution, and the EG individuals, 0.3ml of metronidazole solution, corresponding to 12mg/day in EG I (4%), 18mg/day in EG II (6%), 24mg/day in EG III (8%), 30mg/day in EG IV (10%), and 36mg/day in EG V (12%).

In the third, seventh and 14th days of treatment, six animals of EG I, II, III, IV, V and CG were euthanized by lethal dose of intraperitoneal sodium pentobarbital (120mg/kg), which is the recommended method of euthanasia for rodents and other small mammals, according to Resolution 714 of the Federal Council of Veterinary Medicine of June 20, 2002.

Each animal was placed on a surgical board and photographed by a Cyber-Shot P71, Sony®, 3.2M pixels resolution, maintained on a tripod at a constant distance of 34cm. This procedure was performed in the wound (moment zero) and after euthanasia on the third, seventh and 14th days. The image obtained was imported into the computer software *VeV MDmeasurement Documentation*®, to evaluate the contraction of the wound by digital planimetry. For the calculation of the real area, we used a template provided by the manufacturer of the program as a reference, a square of 3x3cm, positioned to the right side of the wound at the moment of the photograph, which allowed the conversion of the electronic image to a scale in centimeters.

After the photographic records, we resected the wounds with a margin of 1cm of whole skin around the lesion, with depth to the dorsal musculature of the rat. The segment destined for histology was extended onto a paper filter identified and fixed in 10% formaldehyde for 24 hours. After this period, it was submitted to the conventional histological preparation, included in paraffin block and cuts of 5μm.

We used the tissue array method for immunohistochemistry. From the paraffin block, we removed a fragment with a punch number 3 from the central surface area of the wound. The samples taken were deposited in a cassette, according to determination of the map previously elaborated. The material was then sent for immunohistochemical processing using α-SMA and CD34 antibody. The tissue array method allowed the complete analysis of the central area of the wound, with an average of 14 fields evaluated at 400x magnification, considering only the nucleated cells, the research target, ie, the protomyofibroblasts and myofibroblasts.

We used the non-parametric Kruskal-Wallis test to compare the groups at each evaluation moment and to compare the moments of evaluation within each group. Values of $p<0.05$ indicated statistical significance. We analyzed the data using the IBM SPSS v.20.0 software.

RESULTS

Regarding wound contraction, we observed that the areas decreased significantly over time in the CG (p=0.001), in EG I (p=0.001), EG II (p=0.001), EG III (p=0.001), EG IV (p=0.002) and EG V (p=0.002). There was a progressive reduction of the wound on the third, seventh and 14th day in all groups analyzed. In the comparison between the experimental groups and the control one, there was no significant difference in the periods evaluated.

The CD34 immunohistochemistry, used to identify neovascularization, marked stromal cells in the matrix, unrelated to neovessels, α-SMA negative, suggestive of being protomyofibroblasts. The use of the tissue array method allowed the comparison of the slides with α-SMA and CD34 antibodies, ensuring that they were not the same cells. In the evaluation of the third day, none of the groups examined presented protomyofibroblasts in the wounds. On day 14, there was no difference between groups. On the seventh day, there was difference between the groups (p=0.022), according to table 2.

By comparing groups two to two as to the presence of protomyofibroblasts on the seventh day, there was a significant difference, as shown in table 3.

Table 3. *Comparison between groups two to two in relation to the presence of protomiofibroblast on the seventh day.*

Compared groups	p value
Control x 4%	0.096
Control x 6%	0.014
Control x 8%	0.286
Control x 10%	0.258
Control x 12%	0.898
4% x 6%	0.388
4% x 8%	0.532
4% x 10%	0.007
4% x 12%	0.122
6% x 8%	0,142
6% x 10%	0.001
6% x 12%	0.020
8% x 10%	0.033
8% x 12%	0.346
10% x 12%	0.210

Non parametric Kruskal-Wallis test, p<0.05.

With α-SMA immunohistochemistry, at the third day evaluation none of the groups presented myofibroblasts in the wounds. On the seventh day, the difference was not significant. On the 14th day, there was difference between groups (p<0.010), according to table 4.

Table 2. *Number of protomyofibroblasts in the wounds of the control and experimental groups on the seventh day of evaluation.*

	Groups	n	Mean	Standard deviation	p value
Day 7	Control	6	0.612	0.853	0.022
	4%	6	1,602	1,469	
	6%	6	2,355	1,602	
	8%	6	1,589	2,052	
	10%	6	0.140	0.310	
	12%	6	0.481	0.407	

Non parametric Kruskal-Wallis test, p<0.05.

Table 4. *Number of myofibroblasts in the wounds of the control and experiment groups on the 14th day of evaluation.*

	Groups	n	Mean	Standard deviation	p value
Day 14	Control	6	0.186	0.288	0.010
	4%	6	1.061	1,136	
	6%	6	2,530	1,718	
	8%	6	1,287	1,139	
	10%	6	0.981	0.417	
	12%	6	0.235	0,231	

Non parametric Kruskal-Wallis test, p<0.05.

When comparing groups two to two as to the presence of myofibroblasts on the 14th day, there was a significant difference (Table 5).

Table 5. *Comparison between groups two to two in relation to the presence of myofibroblast on the 14th day.*

Compared groups	p value
Control x 4%	0.045
Control x 6%	0.001
Control x 8%	0.021
Control x 10%	0.008
Control x 12%	0,776
4% x 6%	0.084
4% x 8%	0.726
4% x 10%	0.464
4% x 12%	0.081
6% x 8%	0.162
6% x 10%	0.304
6% x 12%	0.001
8% x 10%	0.701
8% x 12%	0.039
10% x 12%	0.016

Non parametric Kruskal-Wallis test, p<0.05.

DISCUSSION

Among the authors who carried out experimental work on rats with open back wounds, healing by secondary intention, and evaluation of wound contraction by planimetry, Prasad *et al*.[12] used of oral metronidazole at the dose of 160mg/kg/day, and Rao *et al*.[13] used it topically at a dose of 180mg/kg/day, both reporting an increase in wound contraction. Conversely, Borden *et al*.[14] used intraperitoneal metronidazole at the dose of 20mg/kg/day, and Trindade *et al*.[4], a topical dose of 50mg/kg/day, and they did not find significant difference in wound contraction when comparing the groups.

Wrobel *et al*.[15] studied cultures of human fibroblasts and myofibroblasts on a contractile substrate and observed similar contractile strengths, suggesting that in the absence of α-SMA expression, the fibroblast could produce sufficient contractile strength for the closure of an open wound. Ibrahim *et al*.[16] evaluated contraction of open wounds in healing by secondary intention in humans and in male and female rats, without drug interference. The authors demonstrated that α-SMA expression of fibroblasts, ie myofibroblasts in granulation tissue, contributed but were not required for wound contraction, and concluded that fibroblasts generate contractile strengths *in vivo*.

Berry *et al*.[17] reported that there was effective contraction of large wounds in the absence of high density myofibroblasts. They have suggested that the contractile unit may be the organization that fibroblasts promote of the thin collagen fibers with the thick ones and the compaction of the connective tissue within the granulation tissue, retracting the dermis and adipose tissue around the wound. In our study, we observed that the wounds of the experimental and control groups had their area significantly decreased as time went by.

Nevertheless, when comparing the groups with each other, there was no difference between times, showing that topical metronidazole at doses of 40, 60, 80, 100 and 120mg/kg/day did not alter the rate of wound contraction by secondary intention healing. Therefore, the rate of reduction of wound size during secondary intention healing was not influenced by metronidazole in topical use, regardless of the doses used.

In the extracellular matrix, there are fibroblasts that present in their cytoplasm bands of microfilaments known as contractile fibers that express actin but are α-SMA-negative, and may be labeled by CD34[1,18,19]. In the early stages of wound healing granulation tissue formation, the fibroblasts around the wound migrate to the center of the lesion and acquire, in their cytoplasms, microfilament bands similar to the contractile fibers beta and gamma actin, becoming protomyofibroblasts, which initiate the synthesis of extracellular matrix components, such as fibronectin and collagen types I and III[18,20]. These immature myofibroblasts appear in the granulation tissue between fifth and sixth days after wound infliction[21].

Protomyofibroblasts secrete a form of fibronectin called ED-A fibronectin, which has been deemed important for the expression of the myofibroblast phenotype in the wound[5,20]. The transformation of the fibroblast into protomyofibroblast seems to depend on the change in the mechanical stress of the wound as compared to the increase in stiffness of the normal skin. However, complete differentiation in myofibroblasts will only occur with the stimulation of the transforming growth factor beta (TGF-β) in the presence of ED-A fibronectin[5,20]. Platelet-derived growth factor (PDGF) and Tumor Necrosis Factor-alpha ply roles on protomyofibroblast formation, but these cytokines isolated fail to induce α-SMA expression and differentiation into myofibroblast *in vitro* or *in vivo*[1,21].

Hinz *et al*.[22], in an experiment with silicon substrate and TGF-β-treated collagen matrix and culture of rat subcutaneous fibroblasts, achieved an increase in α-SMA expression, demonstrating the action of this factor on the contractile activity of fibroblasts. TGF-β acts on the proliferation and differentiation of fibroblasts into myofibroblasts, activation of keratinocytes, matrix deposition and angiogenesis[23].

There are factors that inhibit the expression of α-SMA, such as the basic fibroblast growth factor (bFGF), which antagonizes TGF-β1[24]. Interferon-γ produced by T cells suppresses expression of α-SMA, collagen deposition and contraction in animal models[25]. TGF-β3 alters α-SMA expression, but depends on the cell culture applied[26]. Interleukin-1 antagonizes TGF-β and, after this block, induces myofibroblast apoptosis[27]. The relationship between the extracellular matrix and TGF-β determines normal scarring or a fibrosis process[21,23].

In our study, the experimental groups with the application of 4%, 6% and 8% metronidazole had a higher number of protomyofibroblasts in the wounds. The experimental groups with 10% and 12% presented lower density of these cells than the control group. This suggests that the action of topical metronidazole in the wound in inducing differentiation of the fibroblast into protomyofibroblast is dose dependent.

Knowing the factors of recruitment of fibroblasts and mesenchymal cells, differentiation, proliferation and apoptosis of myofibroblasts are fundamental to understand normal and pathological tissue repair[5]. With regard to myofibroblasts, studies with antibody-labeled immunofluorescence to detect all actin isoforms have shown that these cells originate from the fibroblasts that migrate to the wound[7]. In these studies, myofibroblasts appeared on the sixth day of wound evaluation, and were present from day 12 to day 15. From the 16th to the 20th day, there was an intense decline in the presence of myofibroblasts, and by the 30th day there were no such cells in the wounds. They also observed that fibroblasts apoptotic figures appeared between the 20th and 25th day of the lesion, suggesting that these cells have been programmed to die in cases of wound healing. The authors emphasized that the first phase of wound contraction is independent of the myofibroblast phenotype[7].

Myofibroblasts are cells that have in their cytoplasm bands of organized α-SMA microfilaments. But other markers of muscle fibers, heavy chain myosin, desmin, h-caldesmon and smoothelin are negative[3,8,28]. Tomasek *et al*.[1] stated that the myofibroblast plays roles in the synthesis of the extracellular matrix and in the generation of strength responsible for the reorganization of the matrix and contraction of the wound. The increase in cell line studies and genetic tools has made possible to identify other precursors of myofibroblasts besides fibroblasts from the adjacent intact dermis. Precursors such as vascular smooth muscle cells, pericytes, mesenchymal cells, fibrocytes, stellate hepatic cells, bone marrow cells have been proposed, among others[8,27-29].

According to Hinz *et al*.[21], myofibroblasts are characterized by the development of α-SMA contractile fibers and increased production of extracellular matrix proteins. These cells connect through focal adhesions to the matrix and with each other by adherent junctions (Figure 1). The main cell to originate the myofibroblast after injury to different tissues seems to be the fibroblast residing in the dermis around the lesion, initially differentiating into protomyofibroblast, characterized by being α-SMA negative.

Recent studies have shown that the extracellular matrix develops and maintains tissue homeostasis, and its dysfunction favors the appearance of fibrotic diseases and stromal neoplasms[5,23,30]. Tissue degeneration in malignancy may arise from cases of fibrotic diseases, such as cirrhosis of the liver, pulmonary and renal fibrosis, characterized by hyperproliferation of fibroblasts, their differentiations into myofibroblasts, and abnormal accumulation of collagen[9,21,30]. Knowledge of the complex process of production, modification and remodeling of the extracellular matrix is the key to acting on cellular responses and on antifibrotic and antineoplastic therapies[2,5,9,19,30,31]. Hinz and Gabbiani[32] stated that the myofibroblast is the main cell involved in fibrotic diseases, and the therapeutic strategy would be to interfere in the differentiation of this cell by controlling TGF-β1 and ED-A fibronectin.

There are reports that the mechanical tension in the wound extracellular matrix would be responsible for maintaining the presence of myofibroblasts[33,34]. Considering the possible myofibroblasts precursors, Hinz[35] raises the hypothesis that the myofibroblasts do not display the same response to injuries' mechanical factors, and that probably there are other factors for the maintenance of the myofibroblast phenotype other than change in wound tension.

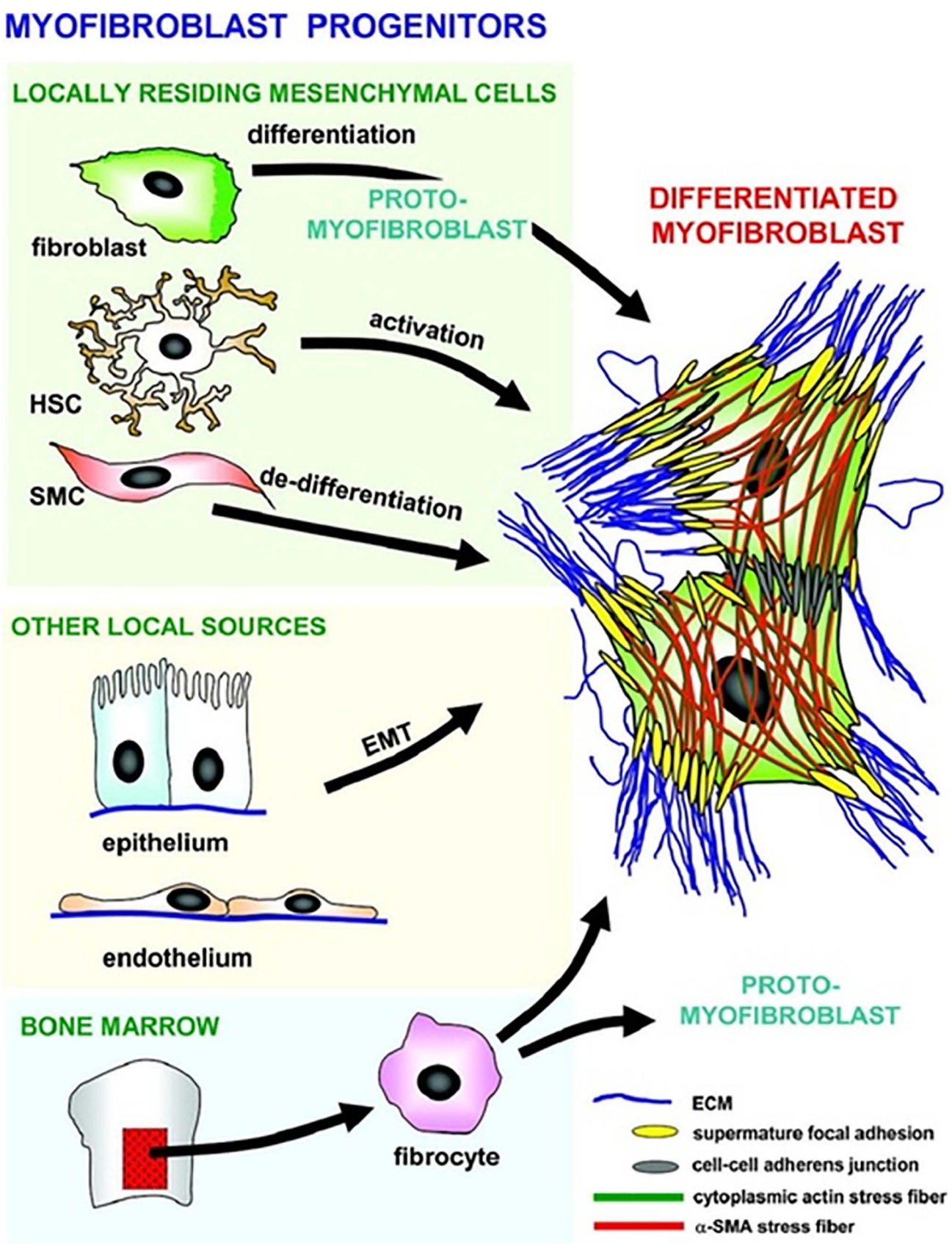

Figure 1. *One cell, multiple origins. Source: Image obtained under license # 3927950953808 from Elsevier's publication in The American Journal of Pathology, The Myofibroblast, volume 170, issue 6, June 2007, in agreement with author Dr. Boris Hinz.*

Protomyofibroblasts and myofibroblasts are present in normal tissues, such as alveolar septum and intestinal crypts, where tissue tension is stable[20].

In the present study, the experimental groups with a topical application of metronidazole solution at 4%, 6% and 8% presented a higher density of myofibroblasts in the wounds, suggesting that the differentiation of protomyofibroblast into myofibroblast, i.e., the expression of α-SMA, depends on the metronidazole dose used. A plausible hypothesis would be that topical metronidazole between 40 and 80 mg/kg/day would increase the action of TGF-ß on the wound, but at high doses, above 100mg/kg/day, it would lead to the blockade of this cytokine necessary for expression of the myofibroblast phenotype, as demonstrated by other studies[21-24]. However, the apparently toxic action of topical metronidazole at doses above 100mg/kg/day in wound healing by secondary intention may be desirable as antifibrotic therapy in the treatment of hypertrophic scarring of the skin or prevention of keloid formation.

Further studies are required to evaluate the action of metronidazole in high doses on the expression of the myofibroblast phenotype, in oral or intravenous use, to determine if doses above 100mg/kg/day by these administration routes would present an equal block of α-SMA expression, thus constituting a form of systemic action antifibrotic therapy, as suggested by Hinz and Gabbiani[32].

We conclude that the topical application of metronidazole in different concentrations on cutaneous wounds healing by secondary intention induces significant proliferation of protomyofibroblasts and myofibroblasts, with maximum effect in the concentration of 6%, but without significantly influencing the wound contraction phase.

R E S U M O

Objetivo: *avaliar os efeitos da administração tópica do metronidazol na diferenciação de fibroblastos e na contração da ferida durante cicatrização experimental por segunda intenção em ratos.* **Métodos:** *cento e oito animais foram submetidos a uma ferida circular no dorso, com 2cm de diâmetro e divididos em seis grupos: grupo controle, com aplicação de solução salina sobre a ferida e cinco grupos experimentais divididos de acordo com a concentração da solução do metronidazol utilizada (4%, 6%, 8%,10% e 12%). Curativos foram realizados diariamente durante todo o período do experimento, que foi subdividido em três momentos de análise: três, sete e 14 dias. A contração da ferida foi avaliada por planimetria digital e os miofibroblastos e protomiofibroblastos foram identificados usando técnicas de imuno-histoquímica CD34 e a-SMA.* **Resultados:** *a contração da ferida não apresentou diferença entre os grupos e o controle. Os protomiofibroblastos foram significativamente mais numerosos aos sete dias (p=0,022) nos grupos metronidazol de 4%, 6% e 8%. Após 14 dias, nos mesmos grupos, os miofibroblastos predominaram significativamente (p=0,01).* **Conclusão:** *a administração tópica de solução de metronidazol em feridas de pele com cicatrização por segunda intenção foi capaz de melhorar a diferenciação de fibroblastos. A fase de contração da cicatrização de feridas permaneceu inalterada, sem redução significativa da contração avaliada pela planimetria digital. Estes resultados podem ser utilizados em favor do processo de cicatrização de feridas.*

Descritores: *Cicatrização. Metronidazol. Administração Tópica. Fibroblastos. Miofibroblastos.*

REFERENCES

1. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol. 2002;3(5):349-63.
2. Hinz B, Phan SH, Thannickal VJ, Prunotto M, Desmoulière A, Varga J, et al. Recent developments in myofibroblast biology: paradigms for connective tissue remodeling. Am J Pathol. 2012;180(4):1340-55.
3. Darby IA, Laverdet B, Bonté F, Desmoulière A. Fibroblasts and myofibroblasts in wound healing. Clin Cosmet Investig Dermatol. 2014;7:301-11.
4. Trindade LC, Biondo-Simões Mde L, Sampaio CP, Farias RE, Pierin RJ, Netto MC. Evaluation of topical metronidazole in the healing wounds process: an experimental study. Rev Col Bras Cir. 2010;37(5):358-63.
5. Darby IA, Zakuan N, Billet F, Desmoulière A. The myofibroblast, a key cell in normal and pathological tissue repair. Cell Mol Life Sci. 2016;73(6):1145-57.

6. Gabbiani G, Ryan GB, Majne G. Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. Experientia. 1971;27(5):549-50.
7. Darby I, Skalli O, Gabbiani G. Alpha-Smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. Lab Invest. 1990;63(1):21-9.
8. Hinz B. Myofibroblasts. Exp Eye Res. 2016;142:56-70.
9. Cox TR, Erler JT. Remodeling and homeostasis of the extracellular matrix: implications for fibrotic diseases and cancer. Dis Model Mech. 2011;4(2):165-78.
10. Da Costa Santos CM, De Mattos Pimenta CA, Nobre MR. A systematic review of topical treatments to control the odor of malignant fungating wounds. J Pain Symptom Manage. 2010;39(6):1065-76.
11. Sampaio CPP, Biondo-Simões MLP, Trindade LCT, Farias RE, Pierin RJ, Martins RC. Inflammatory alterations provoked by metronidazole in wounds: an experimental study in rats. J Vasc Bras. 2009;8(3):232-7.
12. Prasad D, Rao CM. Wound healing profiles of ketorolac, metronidazole and tinidazole administered post-surgically. Indian J Exp Biol. 1995;33(11):845-7.
13. Rao CM, George KM, Bairy KL, Somayaji SN. An appraisal of the healing profiles of oral and external (GEL) metronidazole on partial thickness burn wounds. Indian J Pharmacol. 2000;32(5):282-7.
14. Borden EB, Sammartano RJ, Dembe C, Boley SJ. The effect of metronidazole on wound healing in rats. Surgery. 1985;97(3):331-6.
15. Wrobel LK, Fray TR, Molloy JE, Adams JJ, Armitage MP, Sparrow JC. Contractility of single human dermal myofibroblasts and fibroblasts. Cell Motil Cytoskeleton. 2002;52(2):82-90.
16. Ibrahim MM, Chen L, Bond JE, Medina MA, Ren L, Kokosis G, et al. Myofibroblasts contribute to but are not necessary for wound contraction. Lab Invest. 2015;95(12):1429-38.
17. Berry DP, Harding KG, Stanton MR, Jasani B, Ehrlich HP. Human wound contraction: collagen organization, fibroblasts, and myofibroblasts. Plast Reconstr Surg. 1998;102(1):124-31.
18. Chitturi RT, Balasubramaniam AM, Parameswar RA, Kesavan G, Haris KT, Mohideen K. The role of myofibroblasts in wound healing, contraction and its clinical implications in cleft palate repair. J Int Oral Health. 2015;7(3):75-80.
19. Díaz-Flores L, Gutiérrez R, Díaz-Flores L Jr, Goméz MG, Sáez FJ, Madrid JF. Behaviour of telocytes during physiopathological activation. Semin Cell Dev Biol. 2016;55:50-61.
20. Bochaton-Piallat ML, Gabbiani G, Hinz B. The myofibroblast in wound healing and fibrosis: answered and unanswered questions. F1000Res. 2016 Apr 26;5. pii: F1000 Faculty Rev-752.
21. Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, Gabbiani G. The myofibroblast: one function, multiple origins. Am J Pathol. 2007;170(6):1807-16.
22. Hinz B, Celetta G, Tomasek JJ, Gabbiani G, Chaponnier C. Alpha-smooth muscle actin expression upregulates fibroblast contractile activity. Mol Biol Cell. 2001;12(9):2730-41.
23. Hinz B. The extracellular matrix and transforming growth factor-ß1: tale of a strained relationship. Matrix Biol. 2015;47:54-65.
24. Cushing MC, Mariner PD, Liao JT, Sims EA, Anseth KS. Fibroblast growth factor represses Smad-mediated myofibroblast activation in aortic valvular interstitial cells. FASEB J. 2008;22(6):1769-77.
25. Dooley S, Said HM, Gressner AM, Floege J, En-Nia A, Mertens PR. Y-box protein-1 is the crucial mediator of antifibrotic interferon-gamma effects. J Biol Chem. 2006;281(3):1784-95.
26. Huang T, Schor SL, Hinck AP. Biological activity differences between TGF-ß1 and TGF-ß3 correlate with differences in the rigidity and arrangement of their component monomers. Biochemistry. 2014;53(36):5737-49.
27. Duffield JS, Lupher M, Thannickal VJ, Wynn TA. Host responses in tissue repair and fibrosis. Annu Rev Pathol. 2013;8:241-76.
28. Hinz B. Formation and function of the myofibroblast during tissue repair. J Invest Dermatol. 2007;127(3):526-37.
29. Klingberg F, Hinz B, White ES. The myofibroblast matrix: implications for tissue repair and fibrosis. J Pathol. 2013;229(2):298-309.

30. Pickup MW, Mouw JK, Weaver VM. The extracellular matrix modulates the hallmarks of cancer. EMBO Rep. 2014;15(12):1243-53.
31. Yu H, Mouw JK, Weaver VM. Forcing form and function: biomechanical regulation of tumor evolution. Trends Cell Biol. 2011;21(1):47-56.
32. Hinz B, Gabbiani G. Fibrosis: recent advances in myofibroblast biology and new therapeutic perspectives. F1000 Biol Reports. 2010;2:78.
33. Hinz B, Mastrangelo D, Iselin CE, Chaponnier C, Gabbiani G. Mechanical tension controls granulation tissue contractile activity and myofibroblast differentiation. Am J Pathol. 2001;159(3):1009-20.
34. Hinz B, Gabbiani G. Mechanisms of force generation and transmission by myofibroblasts. Curr Opin Biotechnol. 2003;14(5):538-46.
35. Hinz B. The myofibroblast: Paradigm for a mechanically active cell. J Biomech. 2010;43(1):146-55.

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