Effect of local or systemic hydrocortisone on skin wound healing resistance, in different postoperative periods

Resistência cicatricial cutânea sob efeito de hidrocortisona local ou sistêmica, em distintos períodos pós-operatórios

Luiz Ronaldo Alberti[1], Leonardo de Souza Vasconcellos[2], Andy Petroianu[3]

ABSTRACT

Objectives: To compare the resistance of skin wound healing of mice submitted to local or systemic hydrocortisone administration, in different postoperative periods. **Methods:** An incision and suture was performed on the thoracic skin of 130 male mice: Group 1 (n = 10) resistance of the integer skin; Group 2 (n = 30) submitted only to skin incision and suture; Group 3 (n = 30) skin incision and suture followed by administration of saline fluid; Group 4 (n = 30) skin incision and suture followed by administration of local hydrocortisone; Group 5 (n = 30) skin incision and suture followed by administration of systemic hydrocortisone. The resistance of the wound healing and the weight of the animals were studied on the seventh, 14th and 21st postoperative days. Histologic examination was also performed. **Results:** The mice that received corticoid (Groups 4 and 5) presented significant decreasing on their weight ($p = 0.02$). The Groups 3, 4 and 5 showed lower scar resistance than Group 2 on the seventh postoperative day ($p < 0.05$). On the 14th and 21st days, there was no difference on the skin would healing resistance ($p > 0.05$). **Conclusions:** Administration of hydrocortisone in mice is responsible for weight decreasing and reduction of the skin wound healing resistance during the first postoperative week.

Keywords: Hydrocortisone/administration & dosage; Adrenal cortex hormones/administration and dosage; Wound healing/drug effects; Models, biological; Mice

RESUMO

Objetivos: Comparar a resistência cicatricial cutânea de camundongos submetidos à administração de hidrocortisona por diferentes vias e em distintos períodos pós-operatórios. **Métodos:** Foram estudados 130 camundongos machos submetidos a incisão e sutura de pele da região dorsal do tórax: Grupo 1 (n = 10) resistência da pele íntegra; Grupo 2 (n = 30) incisão da pele e sutura, sem administração de corticóide; Grupo 3 (n = 30) incisão da pele e sutura, seguidas de injeção local de solução salina; Grupo 4 (n = 30) incisão da pele e sutura, seguidas de injeção local de hidrocortisona e Grupo 5 (n = 30) incisão da pele e sutura, seguidas de injeção intra-peritoneal de hidrocortisona. Foram avaliadas a resistência cicatricial, a variação ponderal no sétimo, 14º e 21º dias pós-operatórios, bem como a histologia. **Resultados:** Os camundongos que receberam hidrocortisona tiveram decréscimo ponderal ($p = 0,02$). Quanto à resistência cicatricial da pele, os Grupos 3, 4 e 5 apresentaram valor inferior ao Grupo 2, no sétimo dia pós-operatório ($p = 0,031$). No 14º e 21º dias, não houve diferença entre as tensões cicatriciais. **Conclusões:** A administração de hidrocortisona provoca redução ponderal em camundongos. Sob a ação dessa droga, a resistência cicatricial cutânea é menor na primeira semana pós-operatória, independente da via de administração.

Descritores: Hidrocortisona/administração & dosagem; Corticosteróide/administração & dosagem; Cicatrização de feridas/efeitos de drogas; Modelos biológicos; Camundongos

INTRODUCTION

Skin healing is a complex process that involves inflammation, reepithelization, angiogenesis, granulation tissue formation, and deposition of interstitial matrix, besides other events carried out by different types of cells, such as keratinocytes, fibroblasts, and inflammatory and endothelial cells. These phenomena are influenced by the interstitial matrix, growth factors, and other mediators[1].

It is believed that glucocorticoids hinder the cicatrisation process, likely causing a decrease in cellular proliferation, in neovascularization, and in

Study carried out at the Department of Surgery of Faculdade de Medicina da Universidade Federal de Minas Gerais – UFMG, Belo Horizonte (MG), Brazil.

[1] PhD; Adjunct professor of the Department of Surgery of Universidade Federal de Minas Gerais – UFMG, Belo Horizonte (MG), Brazil.

[2] Graduate student at Universidade Federal de Minas Gerais – UFMG, Belo Horizonte (MG), Brazil.

[3] PhD; Full professor at Universidade Federal de Minas Gerais – UFMG, Belo Horizonte (MG), Brasil.

Corresponding author: Luiz Ronaldo Alberti – Rua Santa Rita Durão, 347 – apto. 301 – Funcionários – CEP 30140-110 – Belo Horizonte (MG), Brasil – Tel.: 31 3223-8656 – e-mail: luizronaldo@zipmail.com.br

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matrix production[2]. In animals, a delay in the afflux of macrophages, neutrophils, and fibroblasts was reported. It is owned that corticosteroids suppress the inflammatory phase of wound healing[2]. It is possible that the chronic use of corticoids influences negatively in reepithelization, neovascularization, and collagen synthesis[3].

The effect of prolonged use of corticotherapy on surgical wound healing shows conflicting results in literature. Several factors participate in this controversy, depending on the type and dosage of corticosteroids used, species of animals studied, duration of treatment, and methods of evaluation of healing efficacy[4]. Diethelm[5] observed that pre- or intraoperative administration of hydrocortisone is associated with a greater incidence of infections and delay in healing time. Rizzo et al.[6] verified that the adverse effects of the adrenocorticotropic hormone (ACTH) and cortisone were dose-dependent. According to Vogel[7], high doses of cortisol and prednisolone reduced the healing resistance of the skin, but small doses, even for a prolonged time, were accompanied by an increase in the healing resistance.

Jalali and Bayat[8], on the other hand, found unspecific effects with moderate and low doses of corticosteroids, whereas Kletsas et al.[9] noted an increased collagen synthesis in fibroblast cultures of human embryo skin by adding small concentrations of corticoids.

The need for further studies in order to understand the effects of corticoids on skin healing motivated this project, which is part of a line of research on wound healing[10-12].

OBJECTIVE

The objective of this study was to compare the skin wound healing resistance after local and systemic administration of hydrocortisone in distinct postoperative periods.

METHODS

This project was carried out according to the recommendations of the international legislations on animal protection and to the Brazilian Animal Experimentation Code (1988). It has been approved by the Chamber of the Department of Surgery of the Faculdade de Medicina da Universidade Federal de Minas Gerais (UFMG) and by the Experimental Research Ethics Committee (CETEA) of the UFMG.

One hundred and thirty male albino CF-1 Swiss mice were used, with an average weight of 40 ± 5 g. The animals were randomly distributed into five groups:

- Group 1 (n = 10): control, not operated;
- Group 2 (n = 30): submitted only to cutaneous incision and suture;
- Group 3 (n = 30): submitted to cutaneous incision and suture, completed by local daily administration of 0.9% saline solution;
- Group 4 (n = 30): submitted to cutaneous incision and suture, completed by daily local administration of hydrocortisone at 10 mg/kg;
- Group 5 (n = 30): submitted to cutaneous incision and suture, completed by daily intraperitoneal administration of hydrocortisone at 10 mg/kg.

In the Control Group, the resistance of intact skin was evaluated. For the study of healing resistance, Groups 2, 3, 4, and 5 were divided into three subgroups each, as per the postoperative periods studied:

- Subgroup A (n = 10): seventh postoperative day;
- Subgroup B (n = 10): 14th postoperative day;
- Subgroup C (n = 10): 21st postoperative day.

The animals were accompanied daily, received the same type and quantity of nutrition, and remained allocated in cages with identical numbers of animals (n = 5).

The operations were conducted under general anesthesia with ketamine hydrochloride (90 mg/kg) and xylazine hydrochloride (10 mg/kg), both intraperitoneally. After shaving the animals' entire dorsal region, the skin of the median dorsal portion of the thorax was incised longitudinally, measuring 3 cm length, sparing the subjacent muscles. Next, the borders of the wound were sutured with four simple stitches, being used 4-0 monofilament polypropylene.

After follow-up time, the animals were euthanized with a lethal inhaled dose of ether after general anesthesia with ketamine hydrochloride and xylazine hydrochloride with the same doses as previously used. The following parameters were studied:

- weight variation of the animals during the follow-up period;
- presence of general and cutaneous postoperative complications;
- measures of rupture tensions of intact skin and cicatrix;
- histological evaluation of the cicatricial tissue

The mice were weighed at the onset of the experiment and immediately after death.

In each animal from Groups 4 and 5, a 0.1 ml solution was injected daily containing hydrocortisone at 10 mg/kg/day, either at the site of the cicatrix (Group 4) or intraperitoneally (Group 5), as of two days before the operation and continuing until evaluation of skin wound healing resistance. In the

group that received only local saline, the volume injected was also 0.1 ml. The application of these injections had the objective of comparing the systemic and local effects of the corticoid, as well as the role of tissue distention at the incision site in the wound healing process.

Resistance of the wound healing was assessed by removing a skin fragment transversal to the scar, measuring 4 x 1 cm, with the cicatrix in its mid portion. The stitches were carefully removed and the fragment was submitted to a tensile resistance testing by electronic tensiometer.

Histological studies were conducted in preparations stained by hematoxylin-eosin and by Gomori's trichrome. The thickness of the cicatricial fibrous neoformation was measured in three different regions: near the cranial extremity, in the mid portion, and near the caudal border.

One-way ANOVA tests were used, followed by the Tukey-Kramer test so the weights and rupture tensions among the groups could be compared. The differences were considered significant for values corresponding to $p < 0.05$.

RESULTS

All mice survived the experiment and progressed satisfactorily. The weight variation of all groups studied is shown on Table 1. The mice that received hydrocortisone, Groups 4 and 5, displayed weight reduction ($p = 0.02$) during the three postoperative periods, while the animals from the Control Group and those that received only saline solution showed increased body weight.

Table 1. Weight variation (mean ± mean standard deviation) of mice at the seventh, 14th and 21st postoperative days

Groups	Weight variation (g)			
	Initial	7th day	14th day	21st day
Group 1	42.4 ± 2.9	–	–	–
Group 2	41.4 ± 2.5	42.2 ± 2.5	43.6 ± 2.4	45.2 ± 2.9
Group 3	41.6 ± 2.2	42.3 ± 2.6	43.5 ± 3.6	44.7 ± 3.1
Group 4	40.9 ± 3.5	35.6 ± 3.4*	37.4 ± 3.1*	39.2 ± 2.8*
Group 5	42.3 ± 2.8	35.5 ± 2.7*	36.8 ± 3.2*	38.8 ± 3.2*

* weight reduction in Groups 4 and 5 as compared to Group 2 ($p = 0.02$; one-way ANOVA and Tukey-Kramer test for multiple comparisons)
Group 1: control, there is only the initial weight since the mice were not operated on; Group 2: only surgery; Group 3: surgery + local injection of 0.9% saline solution; Group 4: surgery + daily local injection of hydrocortisone; Group 5: surgery + daily systemic injection of hydrocortisone

The results of mean cicatricial tension are shown on Table 2. Mice from Groups 3, 4, and 5 displayed values lower than those of Group 2 only on the seventh postoperative day ($p = 0.031$). During the other periods, there were no differences in cutaneous cicatricial resistance in the presence or absence of hydrocortisone.

Table 2. Skin wound healing resistance (mean ± mean standard deviation) of mice at the seventh, 14th and 21st postoperative days

Groups	Healing resistance (g/cm²)			
	Initial	7th day	14th day	21st day
Group 1	1045.8 ± 55.2*	–	–	–
Group 2	–	231.0 ± 9.5**	461.0 ± 8.2	745.5 ± 7.4
Group 3	–	141.2 ± 7.3	457.3 ± 7.1	726.3 ± 10.3
Group 4	–	138.6 ± 8.3	453.8 ± 8.5	720.4 ± 9.8
Group 5	–	147.5 ± 6.6	448.5 ± 9.2	735.3 ± 10.6

*comparing rupture tension of intact skin in Group 1 and rupture tension at the 21st postoperative day in other groups ($p < 0.001$); **comparison between Group 2 and the other groups at the seventh postoperative day ($p = 0.031$)
Group 1: control; Group 2: only surgery; Group 3: surgery + local injection of 0.9% saline solution; Group 4: surgery + daily local injection of hydrocortisone; Group 5: surgery + daily systemic injection of hydrocortisone

Administration of corticosteroid, whether local (Group 4) or systemic (Group 5), did not interfere in cicatricial resistance when compared to Group 2.

In all mice that were operated (Groups 2, 3, 4, and 5), the healing resistance on the seventh postoperative day was lower than that found on the 14th and 21st postoperative days ($p < 0.05$). On the 14th postoperative day, the resistance was lower than that found on the 21st day ($p < 0.05$). Even after 21 days, the healing resistance was lower than that of the intact skin in all groups ($p < 0.01$).

With the macroscopic study of the healing process, on the seventh postoperative day, in Groups 4 and 5, it was verified that the cicatricial area was looser. In the other groups, on the other hand, healing was firmer. There was no infection or other cicatricial anomaly.

Upon histological assessment, it was verified that in Groups 2 and 3, on the seventh postoperative day there was an increase in collagen fibers, presence of an inflammatory infiltrate composed of polymorphonuclear cells, plasmocytes, lymphocytes, and macrophages, besides vascular congestion. In Groups 4 and 5, still during the seventh day, a more heterogeneous pattern was identified, with an inflammatory reaction in addition to the smaller number of fibroblasts and collagen bundles. On the 14th postoperative day, in all the groups, a more homogeneous morphological pattern was observed relative to the first week, with a better structural arrangement of fibroblasts interspersed with firmer fibers that are more ordered. There were no differences between the animals that received and did not receive hydrocortisone., On the 21st postoperative day, on the other hand, collagen bundles were thicker in comparison to the two initial weeks, which shows a more ordered and parallel distribution, with no differences in the aspects of the four groups evaluated.

DISCUSSION

Factors that affect cicatrization are continually researched, and cicatricial delay is one of the frequently

discussed and controversial effects of corticosteroids. Although there are several experimental studies on the relation between healing phenomena and the use of corticosteroids, methodological multiplicity makes its evaluation difficult[2-5].

The mouse was chosen as the experimental animal due to ease of acquisition, handling, accommodations, resistance to surgical aggression, and low mortality shown during infectious processes. Only adult males were used to avoid the hormonal variations of the estrus cycle of females, which could interfere in the tissue repair mechanism[13].

The choice of hydrocortisone dose for this study was based on previous studies that verified that 10 mg/kg/day is the minimal concentration necessary to affect wound healing, and it is also proportional to doses prescribed in several clinical treatments[10-11]. Following orientation given by other authors[7,13], the administration of the drug was initiated in the preoperative phase and continued until the day the animals were euthanized. According to literature[7-8], when corticosteroids are administered before surgery and maintained postoperatively, their harmful effects of them are more evident.

The weight reduction of mice submitted to corticoids is also a known fact. According to previous studies[3,14], corticotherapy used in animals participates in a complex metabolic process that results in malnutrition. Even though no satisfactory explanation has been found for this phenomenon, this worsening of the animal general condition may have contributed to delayed healing. Contrary to some studies[3,8] in which there has been weight loss and interference in cicatrization after seven weeks, in this study, the follow-up period of the mice was a lot shorter than those that had been described, and there was a reduction in weight and healing resistance as early as the first week of corticoid administration.

This result may be due to the delay in collagen deposition, with a consequent reduction of healing resistance. During the first week, all groups displayed a healing resistance lower than that found posteriorly. In the beginning, the cicatricial tissue is still very thin and resistance is low, which hinders evaluation of the hydrocortisone effect. Even so, it was noted that the groups treated with this drug had a lower healing resistance than the Control Group. During the subsequent periods, the healing resistance of all groups was organized, and there was no difference related to the medication.

As to administration route, it is known that in cicatricial processes, topical corticotherapy tends to be utilized more than systemic administration, especially because of its milder side effects. Nevertheless, in this study, the systemic effects of corticosteroids did not differ from the local effects. It is possible that the comparison of an injection of corticosteroid with its use as an ointment might produce different findings, but it is still difficult to obtain results experimentally.

The immediate inflammatory response to trauma is characterized by the presence of hemorrhage, edema, vascular congestion, and inflammatory infiltrate, lasting, on average, for five days. Posteriorly, the fibroplasia phase is accompanied by vascular neoformation and proliferation of fibroblasts, extending, on average, until the 14th day. Histology revealed a delay in the healing of the mice that received hydrocortisone. On the seventh postoperative day, the Control Group was already in the fibroplasia phase, with intense population of fibroblasts and deposition of collagen fibers, while the groups receiving corticosteroid were still in the inflammatory phase, with scarce fibroblastic cells and thin disarrayed collagen fibers[13]. After the second postoperative week, the cicatricial process of all animals was better organized, with no differences among the groups, displaying well-formed connective tissue rich in ordered collagen fibers.

Despite the observation of a decrease in skin wound healing resistance in mice receiving corticoids during the first postoperative week, it is not possible yet to explain its pathophysiology. The immediate inflammatory response to trauma is characterized by the presence of hemorrhage, edema, vascular congestion, and inflammatory infiltrate, lasting about five days. Later, the fibroplasia phase is accompanied by vascular neoformation and fibroblast proliferation, extending, on average, until the 14th day. Finally, the maturation cycle, which can last up to two years, arranges the collagen and devascularizes the granulation tissue[4].

The action of the corticosteroid may interfere in any one of these processes. According to some authors, this drug promotes stabilization of the lysosome membrane, protecting it against its lysis, and consequently reducing the initial inflammatory reaction[4-5,7]. In antagonizing angiogenesis, these drugs inhibit fibroblast proliferation, reducing collagen synthesis[3-4]. This action could possibly explain the reduction in cicatricial tension, but larger studies are still necessary to clarify this issue.

The transposition of the experimental findings to clinical practice should be made with caution. Riobó et al.[15], analyzed patients with intestinal inflammatory disease under treatment with systemic corticotherapy and submitted to surgical treatment, and did not observe changes in complications of ileorectal anastomoses. Fleshner et al. [16], on the other hand, verified postoperative adverse effects in patients using corticoids.

CONCLUSIONS

According to the results of this study, there has been a decrease of weight variation and a reduction of skin wound healing resistance during the first postoperative week in mice submitted to the effects of hydrocortisone.

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