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ORIGINAL ARTICLE

Postoperative pain behavior differs according to type of stimulus in rats

Comportamento em dor pós-operatória varia conforme o tipo de estímulo em ratos

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ABSTRACT

BACKGROUND AND OBJECTIVES: This study addressed the role of skin and deep tissue incisions in two different models of postoperative pain in rats, plantar incision and gastrocnemius incision models.

METHODS: After approval from the Ethics Committee of the institution, male Wistar rats were used in two different experiments. One group of animals was used in the plantar incision model and divided in three groups: sham incision, skin incision and skin and muscle and fascia incision (deep tissue incision). Another group was used in the gastrocnemius incision model and divided into three other groups: sham incision, skin incision and muscle and fascia incision. The animals in the plantar incision model were assessed for paw withdrawal threshold, and the time spent by the animals in the gastrocnemius incision model in the running wheel was recorded

RESULTS: Skin and skin + deep tissue plantar incisions increased hyperalgesia after mechanical stimulus in the paw. Hyperalgesia lasted in the skin group until the 3rd postoperative day (POD), in the skin + deep tissue incision group, hyperalgesia lasted until the 4th POD. Skin and skin + deep tissue groups were significantly different on the 1st, 2nd, 3rd and 4th POD. Time spent in the running wheel was lower in the skin + deep tissue group on the 1st and 2nd POD.

CONCLUSION: Pain behavior elicited after mechanical stimulus in skin and deep tissue incision is more intense than skin incision in the plantar incision model, however, skin and skin + deep tissue incision elicited the same behavior in gastrocnemius incision model

KEYWORDS: Pain, Fascia, Hyperalgesia, Male, Postoperative, Rats, Skin, Wistar.

RESUMO

JUSTIFICATIVA E OBJETIVOS: Este estudo abordou o papel das incisões na pele e nos tecidos profundos em dois modelos diferentes de dor pós-operatória em ratos, modelos de incisão plantar e incisão do gastrocnêmio

MÉTODOS: Após a aprovação do Comitê de Ética da instituição, ratos Wistar machos foram usados em dois experimentos diferentes. Um grupo de animais foi usado no modelo de incisão plantar e dividido em três grupos: incisão sham, incisão na pele e incisão na pele e músculo e fáscia (incisão em tecido profundo). Outro conjunto foi usado no modelo de incisão de gastrocnêmio e dividido em três grupos: incisão sham, incisão na pele e incisão no músculo e fáscia. Os animais no modelo de incisão plantar foram avaliados quanto ao limiar de retirada da pata, e nos animais do grupo de incisão do gastrocnêmio, o tempo gasto pelos animais na roda giratória foi registrado.

RESULTADOS: As incisões plantares na pele e na pele + tecido profundo aumentaram a hiperalgesia após estímulo mecânico na pata. A hiperalgesia durou no grupo pele até o 3º dia pós-operatório (DPO), no grupo pele + incisão em tecido profundo, a hiperalgesia durou até o 4º DPO. Os grupos pele e pele + tecido profundo foram significativamente diferentes entre si no 1º, 2º, 3º e 4º DPO. O tempo gasto na roda de corrida foi menor no grupo pele + tecido profundo no 1º e 2º DPO.

CONCLUSÃO: O comportamento de dor provocado após estímulo mecânico na pele e incisão em tecido profundo é mais intenso do que na incisão da pele no modelo de incisão plantar, no entanto, incisões na pele e na pele + incisão em tecido profundo provocaram o mesmo comportamento no modelo de incisão no gastrocnêmio.

DESCRITORES: Dor, Fáscia, Hiperalgesia, Macho, Pós-operatória, Ratos, Pele, Wistar.

HIGHLIGHTS

Acute postoperative pain is still very prevalent

The mechanisms need to be better studied

The roles of muscles and fasciae are very important in post-operative pain

INTRODUCTION

Postoperative pain (POP) is a common form of acute pain, an expected symptom after large and medium-sized surgeries. Pain begins with tissue damage that occurs during surgery[1]. It is estimated that 313 million surgeries occur worldwide each year, raising the dimension of the issue of POP[2]. Around 78.2% of American patients undergoing surgery have moderate or severe pain on the first postoperative day[3].

POP mechanisms are still under study, it is known that sensitization of primary afferent neurons, of second-order neurons in the dorsal horn of the spinal cord, tissue hypoxia, activation of the TRPA1, phosphorylation of intracellular signaling proteins in nerve terminals after plantar incision drive sensitization of primary afferent neurons and satellite glia cells in dorsal root ganglion[4-8]. The contribution of injured tissues also seems to be different, with a more relevant role being attributed to fascia and muscle injury than to skin injury in pain[5,6]. The present work evaluated the roles of skin and fascia and muscle in painful behaviors in two models of incisional pain, plantar incision and gastrocnemius incision in rats.

METHODS

The study was approved by the Research Ethics Committee of the University of São Paulo Medical School (FMUSP) under the number 130/10. The animals were treated in accordance with the IASP Research Committee Guidelines and Ethical Issues[8]. The animals were housed individually in appropriate polyethylene cages, under controlled environmental conditions, with free access to water and food

Male Wistar rats weighing between 250 and 350 g from the breeding facilities of the FMUSP were used in the experiments. The effects of skin incision versus skin+ deep tissue (fascia and muscle) incisions were evaluated. Two experiments were performed: paw withdrawal threshold using the Randall-Selitto test after skin or skin + plantar muscle and fascia in rat paw, and time spent by animals in the running wheel after skin versus skin, gastrocnemius muscle and fascia incisions.

Plantar incision

The plantar incision was performed as described by reference authors[9] under general anesthesia with 2% isoflurane. The plantar aspect of the right hind paw was prepared with povidone iodine and a 1.0 cm longitudinal incision was made 0.5 cm from the end of the heel with a number 11 blade. The depth of the incision reached the plantar muscle, which was incised, elevated and had its insertion maintained. In the group with skin incision, the plantar muscle was not approached. After hemostasis by digital pressure, the surgical wound was sutured with 5-0 mononylon thread and the anesthetic supply was suspended

Gastrocnemius incision

Under general anesthesia with 2%-4% isoflurane, trichotomy was performed on the posterior part of the animal's right hind paw, antisepsis was performed with povidone iodine and a 2 cm incision was made with a scalpel with an 11 blade in the skin of the posterior region of the rat's hind paw or skin and a longitudinal incision was made in the gastrocnemius muscle and fascia. After hemostasis performed with mechanical compression with gauze, the skin was closed with three simple stitches with 4-0 mononylon thread. After surgery, the animals were placed in their cages. The sutures were removed under general anesthesia with isoflurane at the end of the 2nd POD.

Paw withdrawal threshold

The assessment of paw withdrawal threshold using the Randall-Selitto test was performed in animals submitted to plantar incision. The rats were evaluated before surgery, 1st, 2nd, 3rd, 4th, 7th, 8th and 10th POD. The threshold was measured in grams.

Time spent on the running wheel

The time that the rats remained moving on the running wheel every 24 h was evaluated before the incision and on the 1st, 2nd, 3rd, 4th and 5th POD. Time was measured in seconds.

Experimental groups

A total of 53 male Wistar rats, weighing between 250 and 350g, were used in the experiments, divided into groups, composed of a minimum of eight and a maximum of 10 animals, evaluating POP using in the plantar incision model (PI experiment) in 25 rats. Twenty-eight rats were used in the gastrocnemius incision model (GI experiment). Among the rats in the PI experiment (25), eight were used as sham (S), eight underwent skin incision (Sk) and nine animals underwent skin + deep tissue incision (Sk+DI). In the GI experiment eight animals belonged to the sham group (S), ten underwent skin incision (Sk) and ten underwent skin + deep tissue incision (Sk+DI).

Statistical analysis

As for the statistical data, the response variable, of a continuous nature, was presented by means and standard deviations in each group (type of incision) and at each postoperative moment.

To assess the effect of the type of incision, a mixed linear regression model was used, with a fixed effect of treatment interaction with the postoperative period and with a random effect of the repetition of the sample unit over time. Multiple comparisons were performed between groups for each postoperative time. A significant level of 5% was used. The mixed models were built using the lmer function of the lme4 package and the multiple comparisons with the lsmeans function. The analyses were conducted using the R 4.1.0 (2021) software.

RESULTS

Plantar incision experiment (plantar incision model)

The paw withdrawal thresholds after the application of pressure were presented by their means and standard deviations, in each follow-up period and according to the study groups (Table 1).

Paw withdrawal thresholds after mechanical stimulus were different among the groups. The estimated mean difference of the groups can be better seen in Figure 1, and Table 2 shows the differences among the groups.

Plantar incisions (skin + deep tissue incision or skin) led to evoked hyperalgesia after mechanical stimulus in the paw. Hyperalgesia lasted in the skin group until the 3rd POD, while in the Sk+DI group, the hyperalgesia lasted until the 4th POD. There was also a statistical difference between the Sk and Sk+DI groups on the 1st, 2nd, 3rd and 4th POD (Table 2). In the remaining time points, there was no difference between the groups that received incisions and the sham group.

GI experiment (gastrocnemius incision model)

Time spent by animals in the wheel also varied but less between the groups with incision, and are shown in Tables 3, 4 and Figure 2.

There was a significant decrease in the time spent by the animals in the running wheel compared to the sham group only in the Sk+G group, on the 1st and 2nd POD. There was no difference between the sham group and the skin group. Between the Sk+G and Sk groups, there was a marginal difference on the 1st POD ($p=0.071$) (Table 4). The behavior of the groups can be seen in Figure 2 with the estimated means of the groups.

DISCUSSION

POP is still a challenge in daily clinical practice. The roles of different somatic tissues in clinical pain are being studied to explain clinical findings like the fact that POP in patients submitted to total hip arthroplasty using muscle sparing incision feel less pain than patients whose muscles were divided and incised[10]. In another study, there was no difference in POP between groups where the same degree of deep tissue occurred but with different lengths of incised skin[11].

Therefore, the belief is that the muscular component is more important than the skin[4,5,12]. In these studies, the contributions of skin and muscle to POP were studied in the same models of

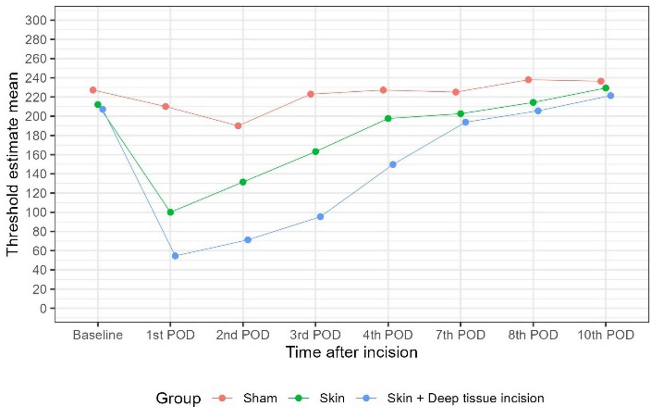

Figure 1. Assessment of estimated means of paw withdrawal thresholds using the (Plantar Incision model) experiment.

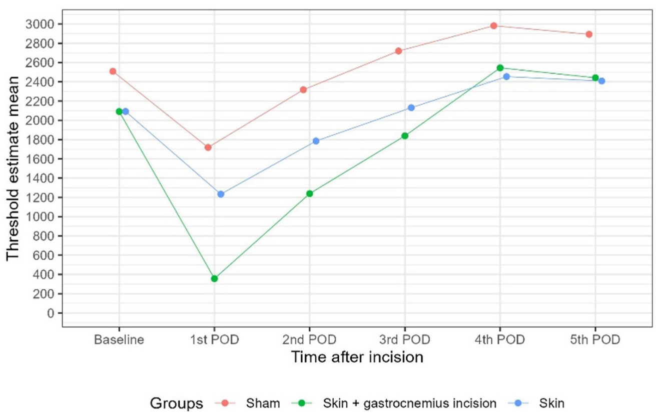

Figure 2. Estimated means of time spent on the running wheel among groups.

Table 1. Initial data description in plantar incision model experiment.

Time	Sham (g)	Skin (g)	Skin + Deep tissue (g)
Baseline	227.2 ± 25.3 (n=8)	212.1 ± 40.3 (n=8)	207.2 ± 27.2 (n=9)
1st POD	210.1 ± 35.5 (n=8)	100.0 ± 13.9 (n=8)	54.6 ± 9.7 (n=9)
2nd POD	190.0 ± 41.2 (n=8)	131.5 ± 43.3 (n=8)	71.2 ± 18.5 (n=9)
3rd POD	223.0 ± 31.4 (n=8)	163.1 ± 43.1 (n=8)	95.3 ± 31.5 (n=9)
4th POD	227.2 ± 38.7 (n=8)	197.6 ± 40.5 (n=8)	149.8 ± 47.8 (n=9)
7th POD	225.1 ± 46.0 (n=8)	202.6 ± 51.1 (n=8)	193.8 ± 45.4 (n=9)
8th POD	238.0 ± 21.4 (n=8)	214.4 ± 47.1 (n=8)	205.7 ± 44.7 (n=9)
10th POD	236.5 ± 17.0 (n=8)	229.4 ± 35.4 (n=8)	221 ± 44.0 (n=9)

Table 2. Multiple comparisons between the estimated means of paw withdrawal thresholds over the postoperative days among the groups.

Comparisons	Time	Estimated mean difference	CI inf.	CI sup.	p-value
Sh - Sk	Baseline	15.12	-21.41	51.66	0.4137
Sh – Sk+DI	Baseline	20.03	-15.48	55.,54	0.2660
Sk – Sk+DI	Baseline	4.90	-30.61	40.41	0.7848
Sh - Sk	1st POD	110.12	73.59	146.66	<0.001
Sh – Sk+DI	1st POD	155.57	120.06	191.08	<0.001
Sk – Sk+DI	1st POD	45.44	9.93	80.95	0.0126
Sh - Sk	2nd POD	58.50	21.96	95.04	0.0020
Sh – Sk+DI	2nd POD	118.78	83.27	154.29	<0.001
Sk – Sk+DI	2nd POD	60.28	24.77	95.79	0.0011
Sh - Sk	3rd POD	59.87	23.34	96.41	0.0016
Sh – Sk+DI	3rd POD	127.67	92.16	163.18	<0.001
Sk – Sk+DI	3rd POD	67.79	32.28	103.30	0.0003
Sh - Sk	4th POD	29.62	-6.91	66.16	0.1109
Sh – Sk+DI	4th POD	77.47	41.96	112.98	<0.001
Sk – Sk+DI	4th POD	47.85	12.34	83.36	0.0087
Sh - Sk	7th POD	22.50	-14.04	59.04	0.2249
Sh – Sk+DI	7th POD	31.35	-4.16	66.86	0.0830
Sk – Sk+DI	7th POD	8.85	-26.66	44.36	0.6224
Sh - Sk	8th POD	23.62	-12.91	60.16	0.2027
Sh – Sk+DI	8th POD	32.33	-3.18	67.84	0.0739
Sk – Sk+DI	8th POD	8.71	-26.80	44.22	0.6278
Sh - Sk	10th POD	7.12	-29.41	43.66	0.6998
Sh – Sk+DI	10th POD	15.06	-20.45	50.57	0.4025
Sk – Sk+DI	10th POD	7.93	-27.58	43.44	0.6588

Sh = sham; Sk = skin incision; DI = deep tissue incision; CI: Conficence Interval.

Table 3. Initial data description in the gastrocnemius incision model experiment.

Time	Sham	Skin incision	Skin + gastrocnemius incision
Baseline	2508.8±1585.7 (n=8)	2092.3±754.7 (n=10)	2091.3±616.4 (n=10)
1st POD	1718.5±856.2 (n=2)	1233.5±1076.8 (n=10)	356.6±226.8 (n=10)
2nd POD	2317.1±1216.0 (n=8)	1785.2±847.1 (n=10)	1239.2±559.4 (n=10)
3rd POD	2719.6±1370.3 (n=8)	2131.3± (n=10)	1838.5±1166.8 (n=10)
4th POD	2981.9±1442.3 (n=8)	2453.6±950.1 (n=10)	2544.7±1179.2 (n=10)
5th POD	2893.4±1344.9 (n=8	2408.3±895.5 (n=10)	2441.5±1073.0 (n=10)

incisional pain that were used in the present study: the plantar incision model and the gastrocnemius incision model, and observed pain behaviors after incisions made on skin or on skin, fascia and muscle (deep incision), showed the occurrence of secondary hyperalgesia after mechanical stimulus, but not after heat stimulus in the incisions that involved the muscle.

In the present study's experiments, mechanical hyperalgesia occurred after skin incision and after skin plus deep tissue incision,

Table 4. Multiple comparisons between estimated mean time spent on the running wheel during the postoperative days among groups.

Comparisons	Time	Estimated mean difference	CI inf.	CI sup.	p-value
Sh – Sk+G	Baseline	417.45	-596.16	1431.06	0.4103
Sh - Sk	Baseline	416.45	-597.16	1430.06	0.4114
Sk – Sk+G	Baseline	-1.00	-956.64	454.63	0.9983
Sh – Sk+G	1st POD	1316.90	248.29	2375.51	0.0097
Sh - Sk	1st POD	485.00	-528.61	1498.61	0.3395
Sk – Sk+G	1st POD	-876.90	-1832.54	78.74	0.0711
Sh – Sk+G	2nd POD	1077.93	64.31	2091.54	0.0377
Sh - Sk	2nd POD	531.93	-481.69	1545.54	0.2954
Sk – Sk+G	2nd POD	-546.00	-1501.64	409.64	0.2552
Sh – Sk+G	3rd POD	881.13	-132.49	1894.74	0.0866
Sh - Sk	3rd POD	588.33	-425.29	1601.94	0.2478
Sk – Sk+G	3rd POD	-292.80	-1248.44	662.84	0.5394
Sh – Sk+G	4th POD	437.18	-576.44	1450.79	0.3887
Sh - Sk	4th POD	528.28	-485.34	1541.89	0.2986
Sk – Sk+G	4th POD	91.10	-864.54	1046.79	0.8483
Sh – Sk+G	5th POD	451.88	-561.74	1465.49	0.731
Sh - Sh+Sk	5th POD	485.08	-528.54	1498.69	0.3394
Sk – Sk+G	5th POD	33.20	-922.44	988.84	0.9444

Sh = sham; Sk = skin incision; SK+G = skin and gastrocnemius incision; CI: Conficence Interval.

returning to basal values as the days progressed. After wounding the plantar muscle was more intense and lasted longer than the pain of wounding only skin. The withdrawal threshold was lower in the Sk+DI compared to sham and skin groups in the first four postoperative days, similar to previous results[5].

In the GI experiment, there was a decrease in the time spent by the animals in the wheel in the 1st and 2nd POD in the Sk+G group compared to the sham group, but there was no difference between Sk and Sk+G, only a tendency of shorter time spent by animals of the Sk+G group compared to Sk group in the running wheel during the 1st POD.

The present study confirmed previous results that showed that the role of fascia and muscle is more important than incision in POP and proposed two hypotheses to explain the fact that the muscular component had more importance in the plantar incision model, and less importance in the gastrocnemius incision model. In the plantar incision model, the pain component evoked was more intense, the painful stimulus was applied directly on the incision and the sensitive innervation was more present in the plantar part of the paw than in more proximal regions of the inferior limb. In the running wheel, deambulation utilizes the gastrocnemius muscle, however, the nociceptive stimulus was not applied directly on the muscle. Another possibility, arising from the tendency observed in the 1st POD, is that the sample size was not adequate for this model of incisional pain so that difference could have been perceived.

CONCLUSION

The study showed the relevance of muscle in the genesis of postoperative somatic pain, but this role may vary according to the muscular group injured and/or of the nociceptive stimulus used.

Lastly, differences were observed in both of the models between animals with skin, skin and fascia and muscle incisions, where it is observed that the deeper the incision, the higher the sensitization, which demonstrates that muscle activation or deep tissues sensitizes more nociceptors. In numerous data available, in addition to many randomized clinical essays suggest that mechanical hyperalgesia lasting several days has some similarities to the state of human POP. These will permit a deeper investigation of the components of incisional pain and therefore test new therapies for its treatment.

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Omar Ashmawi: Research, Writing - Preparation of the original
Eduardo Pio Cunha: Writing - Preparation of the original, Writing - Review and Editing
Danilo Ramirez De-Gregori: Writing - Preparation of the original, Writing - Review and Editing
Hazem Adel Ashmawi: Statistical Analysis, Conceptualization, Writing - Preparation of the original, Supervision