УДК 616-018-001.32:615.031:547.995.15]-07-092.9
https://doi.org/10.20538/1682-0363-2022-2-137-144

Screening of local hyaluronic acid injection modes to increase the efficiency of treating crush injury of soft tissues

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ABSTRACT

Aim. To study the state of microcirculation and metabolic activity of the soft tissues in the compression area in experimental crush injury after local hyaluronic acid injection and to determine the effective mode of its application.

Materials and methods. The experiments were carried out on 178 male Wistar rats aged 4–4.5 months and weighing 280–340 g. The study design included anesthesia, modeling of crush injury (CI), local injection of 1.75% hyaluronic acid (HA) solution into the compression area, systemic intravenous injection of 0.9% sodium chloride solution daily for 3 days, and a study of microcirculation and metabolism of the soft tissues in the damaged area 3, 7, 14, and 28 days after the injury.

Results. Early (3 hours after the injury) local application of HA for CI improved microcirculation, increased oxygen consumption, and activated oxidative metabolism in the skeletal muscles, which helped reduce the severity of destructive processes in the damaged area. The most effective injection mode was two-fold administration of HA: 3 hours after the compression cessation and additionally 24 hours after the injury.

Conclusion. In the crush injury, early local intramuscular injection of HA into the damaged area in the first few hours after the cessation of compression is a sanogenetically substantiated method for correcting traumatic ischemia of the muscles.

Keywords: crush injury, traumatic ischemia of the muscles, microcirculation, metabolic state, hyaluronic acid, laser Doppler flowmetry

Conflict of interests. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

Source of financing. The authors state that they received no funding for the study.

Conformity with the principles of ethics. The study was approved by the local Ethics Committee at the State Scientific Research Testing Institute of Military Medicine (Protocol No. 13 of 22.06.2020).

For citation: Shperling I.A., Shulepov A.V., Shperling N.V., Kuzmina O.Yu. Screening of local hyaluronic acid injection modes to increase the efficiency of treating crush injury of soft tissues. *Bulletin of Siberian Medicine.* 2022;21(1):137–144. https://doi.org/10.20538/1682-0363-2022-2-137-144.

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Скрининг режимов локального применения гиалуроновой кислоты для повышения эффективности лечения компрессионной травмы мягких тканей

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РЕЗЮМЕ

Цель. Изучить состояние микроциркуляции и метаболической активности в мягких тканях области компрессии при экспериментальной компрессионной травме после локального введения гиалуроновой кислоты и определить эффективный режим ее применения.

Материалы и методы. Эксперименты выполнены на 178 самцах крыс линии Вистар весом 280–340 г в возрасте 4–4,5 мес. Дизайн исследования включал в себя обезболивание, моделирование компрессионной травмы (КТ), локальное введение 1,75%-го раствора гиалуроновой кислоты (ГК) в область компрессии, системное внутривенное введение 0,9%-го раствора натрия хлорида ежедневно 3 сут, исследование микроциркуляции и метаболизма мягких тканей области повреждения через 3, 7, 14 и 28 сут после травмы.

Результаты. Раннее (через 3 ч после травмы) локальное применение ГК при КТ улучшает микроциркуляцию, повышает потребление кислорода, активирует окислительный метаболизм скелетных мышц, что способствует уменьшению выраженности деструктивных процессов в области повреждения. Наиболее эффективным является двукратное введение ГК через 3 ч после прекращения компрессии и дополнительно через 24 ч после травмы.

Заключение. При компрессионной травме мягких тканей раннее локальное внутримышечное введение гиалуроновой кислоты в область повреждения в первые несколько часов после прекращения компрессии является саногенетически обоснованным способом коррекции ишемических повреждений.

Ключевые слова: компрессионная травма, травматическая ишемия мышц, микроциркуляция, метаболизм, гиалуроновая кислота, лазерная допплеровская флоуметрия

Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Соответствие принципам этики. Исследование одобрено локальным комитетом по этике ФГБУ «ГНИИИ ВМ» МО РФ (протокол № 13 от 22.06.2020).

Для цитирования: Шперлинг И.А., Шулепов А.В., Шперлинг Н.В., Кузьмина О.Ю. Скрининг режимов локального применения гиалуроновой кислоты для повышения эффективности лечения компрессионной травмы мягких тканей. *Бюллетень сибирской медицины.* 2022;21(2):137–144. https://doi.org/10.20538/1682-0363-2022-2-137-144.

INTRODUCTION

Crush injury (CI) (or traumatic ischemia of muscles) is a pathological process that develops after prolonged pressure on soft tissues, mainly skeletal muscles of the extremities, leading to the destruction of muscle fibers and formation of extensive defects of muscle tissue with their subsequent replacement with connective tissue [1]. For normal functioning of skeletal muscles under physiological conditions, as well as during their regeneration after injury, the state of local microcirculation, which provides the metabolic demand for oxygen and nutrients for muscle tissue, is of particular importance [2]. High microcirculation in the muscles stimulates proliferation of myoblasts and facilitates migration of other cells to the injured area [3]. The creation of local matrix-mediated conditions for intercellular interactions promotes activation of a cambial reserve of muscle tissue [4].

One of the promising approaches is the use of biodegradable hydrogels based on hyaluronic acid (HA),

which induce proliferation and migration of poorly differentiated cells [5]. The positive regenerative effect of HA during local application is associated with improved microcirculation, activation of metabolism, and increased angiogenesis and reparative myogenesis [6]. Even a single local injection of HA in the early post-injury period contributes to the restoration of microcirculation and activation of metabolism in the skeletal muscles of the injured area [7]. To determine the therapeutic potential of HA and to identify the mechanisms of its effect on skeletal muscle regeneration in CI, studying the effects of HA on microcirculation and tissue metabolism of the injured area after its single and multiple administration at different times of the post-injury period is required. This will make it possible to develop a method for the local application of HA which will help improve the techniques for treating patients with CI.

The aim of the research was to study the state of microcirculation and metabolic activity of the soft tissues in experimental CI of the limb after local injection of HA into the injured area at different times after the injury.

MATERIALS AND METHODS

The experiments were carried out on 178 adult male Wistar rats weighing 310 ± 30 g, obtained from the Rappolovo nursery (Leningrad region, Russian Federation), in the laboratory of the State Scientific Research Testing Institute of Military Medicine. The rats were 4–4.5 months old. Prior to the experiment, all animals were quarantined for 14 days (air temperature 25 ± 2 ° C, free access to food and water). The study was approved by the local Ethics Committee at the State Scientific Research Testing Institute of Military Medicine (Protocol No. 13 of 22.06.2020) and conducted in accordance with the Directive 2010/63/EC, the Declaration of Helsinki, and the "Rules for Conducting Work with the Use of Experimental Animals".

The study design included the following stages: local anesthesia; CI simulation; local injection of HA into the injured area; systemic intravenous administration of 0.9% sodium chloride solution daily for 3 days; study of microcirculation and metabolism in soft tissues of the injured area at day 3, 7, 14, and 28 after the injury.

For local anesthesia before CI simulation, the rats were intramuscularly injected with zoletil (Virbac, France) and xylazine (Pharmamagist Ltd., Hungary) at a dose of 10 mg / kg for each drug. CI modeling was carried out by controlled mechanical compression of the soft tissues of the thigh according to the technique described above [7].

All animals were divided into 6 groups consisting of 28 animals each: 5 main (I–V) groups and a control group. The animals of the main group I (HA-3) were locally injected with a sterile disposable syringe by the fan technique with an aqueous solution of HA in the injured area once 3 hours after the cessation of the compression; in group II (HA-24), HA was injected once after 24 hours; in group III (HA-48), HA was administered once after 48 hours; in group IV (HA-3 + 24), HA was injected twice, after 3 hours and additionally after 24 hours; in group V (HA-3 + 24 + 48), HA was injected three times, after 3 hours and additionally after 24 and 48 hours. The rats of the control group (n = 28) did not receive local treatment. Intact animals (n = 10) were not subjected to compression.

For the study, HA was used in the form of an aqueous solution of Hyalift 3.5 gel (Aestetic Dermal SL, Spain), which was diluted with 0.9% sodium chloride solution in the 1:1 ratio immediately before administration to obtain 1.75% HA solution with the necessary fluidity. HA solution was injected intramuscularly into the compression area of experimental animals using a fan technique in a total volume of 0.5–0.8 ml per animal. In order to prevent dehydration, all animals with CI for 3 days were injected daily into the tail vein with 0.9% sodium chloride solution at a dose of 2.0 ml / kg of body weight.

In the course of dynamic observation of experimental animals, microcirculation and metabolism in the skeletal muscles of the thigh (pelvic) limb region were assessed using the LAKK-M complex (SPE LAZMA, Russian Federation) at days 3, 7, 14, and 28 after the injury. Under anesthesia, a skin incision was made in the compression area, and a measuring sensor of the device was placed on the thigh muscles. The duration of measurement was 10 min, the depth of probing the muscle tissue volume was 1.0–1.5 mm. In the Laser Doppler Flowmetry (LDF) mode of the device, the amplitude of the parameters M and σ (constant and variable components of microcirculation) was measured; the coefficient of variation (K_v, %) was calculated using the formula: $K_v = \sigma / M \times 100\%$. An increase and (or) decrease in the K_v value indicated improvement and (or) deterioration in the state of microcirculation, respectively.

In the Optical Tissue Oximetry (OTO) mode of the device, the parameter of blood oxygen saturation in the microvasculature of the probed biological tissue

(SO_2, %) was measured, and the index of specific oxygen consumption in the tissue (*U*, relative units) was calculated. The metabolic rate in the skeletal muscles in the injured area was measured in the Laser Fluorescence Diagnosis (LFD) mode, and the amplitude of the fluorescence spectra of the reduced form of nicotinamide adenine dinucleotide (N_{AD}, relative units) and the oxidized form of flavin adenine dinucleotide ($_{FAD}$, relative units) was determined. After that, we calculated the oxygen consumption rate (OCR, relative units) according to the formula: OCR = $_{FAD}$ / N_{AD}. The complex state of microcirculation and metabolism of the skeletal muscles was assessed using the parameter of the efficiency of oxygen metabolism (EOM, relative units), calculated as EOM = M × U × OCR. EOM and OCR are the most informative parameters that characterize the relationship between the state of microcirculation and the intensity of metabolism in tissues.

The levels of myoglobin and potassium in the blood serum were determined using the automated enzyme-linked immunosorbent assay (ELISA) and biochemistry analyzer ChemWell 2910 (Awareness Technology, Inc., USA). An increase in the potassium level indicated the destruction of cells following trauma, while an increase in myoglobin indicated the destruction of muscle tissue [1]. The intact rats were used in the experiment to obtain the average static values of the norm.

The data obtained were processed using the Statistica 10.0 software (StatSoft Inc., USA). After testing the hypothesis for normality of distribution using the Kolmogorov – Smirnov test, the median and the upper / lower quartiles *Me* (Q_{25}-Q_{75}) were calculated. When comparing the data, the nonparametric Mann – Whitney U-test was used. The differences between the values were considered statistically significant at $p < 0.05$.

RESULTS

The death of the animals in the main, control, and comparison groups was observed in the first 4 days and averaged 33%. CT scans showed pronounced microcirculation disorders in the injured area. So, the K_v coefficient in all periods of the follow-up was reduced by 35–49% ($p < 0.05$) relative to the intact rats, with minimum values on days 3–7 after injury. Local circulation disturbances in the injured muscles led to a decrease in tissue oxygen consumption, the SO_2 index initially (on days 3–7) increased by 2.7–2.8 times ($p < 0.05$) and then, by day 28, decreased slightly (by 2.2 times at $p < 0.05$) compared with the intact animals, which indicated high levels of oxygen in the blood that was not metabolized by tissues.

Similar oppositely directed changes were noted in the dynamics of the *U* index. Low oxygen consumption by soft tissues led to disruption of oxidative processes in them. The OCR index on day 3 increased by 90.1% ($p < 0.05$) relative to the values in the intact animals and then recovered on days 7–28. Changes in the EOM parameter were recorded, which was at most reduced by 10.7 times by the end of day 7 ($p < 0.05$) compared with healthy animals. It is possible that the increase in the intensity of metabolic processes in the soft tissues of the injured area in the early periods after the cessation of compression (3 days) was due to the activation of anaerobic metabolism using energy resources (glycogen) located in the preserved muscle fibers. Depletion of these reserves led to a decrease in oxidative processes in tissues against the background of low oxygen consumption by cells.

Local injection of HA in the injured area improved tissue perfusion. The most pronounced positive changes in the studied parameters were observed in the animals that were injected with HA, starting from 3 hours after the cessation of compression (HA-3, HA-3/24, HA-3/24/48), while in the groups of animals with two-fold and three-fold injection, the microcirculation parameters were significantly better. So, the K_v index in the HA-3/24 and HA-3/24/48 groups increased by 38.6–60.3% ($p < 0.05$) compared with the control group during all observation periods and by 9.9–11.2% ($p < 0.05$) compared with the HA-3 and HA-24 groups in the early post-compression period (on days 3–7). HA injection in a later period (48 hours) did not lead to an increase in microcirculation in the injured area. It should be noted that tissue perfusion indices in all the experimental groups showed a positive trend during the entire observation period. According to K_v data, by the end of day 28 in the HA-3/24 and HA-3/24/48 groups, microcirculation in the injured area was restored to values of the intact animals.

Improvement of microcirculation in the injured area contributed to an increase in oxygen consumption by tissues. So, according to SO_2 and *U* data, the intensity of oxygen utilization was the highest in the animals from the HA-3, HA-24, HA-3/24, and HA-3/24/48 groups at all periods of observation. In the groups with two-fold and three-fold injection, the SO_2 parameter on days 3 and 28 was lower by 7.6–16.6% ($p < 0.05$), and the *U* parameter was higher by 20.0% ($p < 0.05$) only on day 28 compared with rats from the HA-3 group. The worst values of oxygen consumption

were observed when HA was injected 48 hours after the cessation of compression. Complete restoration of tissue oxygen saturation was not observed in the compression area after the injection of HA.

All animals from the experimental groups showed pronounced changes in the FAD and NAD amplitude ratio. As a result, OCR in the HA-3 and HA-24 groups was elevated on days 14–28, and in the HA-3/24 and HA-3/24/48 groups – starting from day 7 – by 1.8–2.6 times ($p < 0.05$) compared with the control group. Late use of HA (48 hours after the cessation of compression) led to an increase in metabolism in the early stages (3 days) due to the activation of anaerobic processes and had no significant differences with rats from the control group. Changes in microcirculation, oxygen consumption, and metabolism in the tissues of the compression area were reflected in the dynamics of EOM, which had minimum values on day 7 in all the experimental groups.

The maximum EOM values were observed in the HA-3 and HA-24 groups on days 14–28 and in the HA-3/24 and HA-3/24/48 groups – at all periods of the observation. The use of HA after 48 hours led to a slight increase in EOM only by the end of day 28. Two-fold and three-fold injection of HA significantly increased the efficiency of oxygen metabolism in the injured area (by 37.3–48.2%, $p < 0.05$) on days 14–28 compared with rats from the group with a single early injection of HA after 3 hours. It should be noted that in the rats from the HA-3/24 and HA-3/24/48 groups, the EOM index was restored to the values of the intact animals by the end of the observation period (day 28). Considering that the EOM index reflects the state of tissue perfusion, oxygen saturation, and metabolism, the presented dynamics indicates a positive and (or) negative effect of HA on tissue metabolism with its early and (or) late injection.

Impaired tissue metabolism in CI leads to the development of necrobiotic processes in soft tissues. The breakdown products of damaged muscles enter the systemic circulation with the development of myoglobinemia and hyperkalemia. Early single and (or) two-fold local injection of HA (after 3 and 24 hours) led to a decrease in the myoglobin level by 19.7–38.7% ($p < 0.05$) on days 3–14 compared with the control group. The use of HA at a later time (after 48 hours) did not cause a decrease in myoglobin in the blood. By the end of the observation period, the severity of myoglobinemia decreased, but it remained 4.1 times higher ($p < 0.05$) than in the intact animals. Similar changes were observed in the dynamics of the blood potassium level, which on day 3 decreased on average by 36.8% ($p < 0.05$) in the HA-3/24 group compared with the control group. Later (days 7–28), the concentration of potassium in the blood normalized and was equal to the values in the intact rats.

In Tables 1 and 2, the groups of animals with the best values for microcirculation, oxygen consumption, and oxidative metabolism and with low levels of myoglobin and potassium in the blood serum are at the top of the table; the groups with the worst values are at the bottom.

Table 1

Parameters of microcirculation and metabolism in the soft tissues of the thigh of rats after local injection of hyaluronic acid in different modes in experimental crush injury, *Me* (Q_{25}–Q_{75})

Experimental groups	Observation period after injury, days	*n*	K_v, %	SO_2, %	*U*, rel. units	OCR, rel. units	EOM, rel. units
Intact animals (*n=10*)		10	13.5 (12.5–14.3)	31.6 (30.3–32.9)	3.10 (3.04–3.16)	0.51 (0.43–0.56)	22.5 (21.5–23.6)
Main group IV (HA-3/24), *n = 28*	3	8	10.91^{1-4} (10.4–11.7)	64.1^{1-4} (62.7–65.4)	1.50^{1-3} (1.42–1.66)	0.90^{1} (0.84–0.96)	9.7^{1-3} (8.6–10.9)
	7	8	11.7^{1-4} (11.1–12.3)	78.6^{1-3} (77.5–80.2)	1.22^{1} (1.10–1.30)	$0.63^{2,3}$ (0.57–0.75)	6.1^{1-3} (4.8–7.6)
	14	6	$11.8^{2,3}$ (11.3–12.5)	59.8^{1-3} (58.0–60.6)	1.64^{1-3} (1.59–1.74)	0.79^{1-3} (0.70–0.89)	12.6^{1-4} (11.7–13.8)
	28	6	$12.2^{2,3}$ (11.6–12.9)	39.8^{1-4} (39.0–40.6)	2.46^{1-4} (2.42–2.59)	0.90^{1-3} (0.81–1.00)	22.8^{2-4} (21.6–24.6)
Main group V (HA-3/24/48), *n = 28*	3	8	11.4^{1-4} (10.3–11.8)	63.5^{1-4} (62.1–64.8)	1.54^{1-3} (1.46–1.70)	0.89^{1} (0.85–0.97)	10.1^{1-3} (9.0–11.2)
	7	8	11.8^{1-4} (11.2–12.4)	77.8^{1-3} (76.7–79.4)	1.26^{1} (1.16–1.39)	0.60^{2} (0.52–0.73)	5.8^{1-3} (4.6–7.0)
	14	6	$12.0^{2,3}$ (11.4–12.6)	60.1^{1-3} (59.0–62.1)	1.63^{1-3} (1.58–1.73)	0.81^{1-3} (0.72–0.91)	12.5^{1-4} (11.1–13.8)
	28	6	$12.1^{2,3}$ (11.4–12.7)	40.2^{1-4} (39.4–41.0)	2.44^{1-4} (2.40–2.57)	0.89^{1-3} (0.79–0.94)	21.9^{2-4} (21.3–23.6)

Table 1 (continued)

Experimental groups	Observation period after injury, days	*n*	K_v, %	SO_2, %	*U*, rel. units	OCR, rel. units	EOM, rel. units
Main group I (HA-3), *n = 28*	3	8	9.8[1, 2] (8.7–10.2)	69.4[1-3] (67.5–71.4)	1.38[1, 2] (1.34–1.48)	0.90[1] (0.84–0.98)	8.6[1, 2] (7.5–9.8)
	7	8	10.1[1-3] (9.2–10.5)	79.7[1-3] (77.3–80.9)	1.20[1] (1.10–1.33)	0.50 (0.41–0.58)	4.1[1] (2.7–5.1)
	14	6	10.6[1, 2] (10.0–11.3)	62.1[1-3] (61.0–64.1)	1.58[1-3] (1.55–1.65)	0.77[1-3] (0.70–0.82)	8.5[1-3] (7.5–10.1)
	28	6	11.1[1, 2] (10.4–11.7)	47.7[1-3] (46.8–48.6)	2.05[1-3] (2.02–2.13)	0.89[1-3] (0.79–1.00)	16.6[1-3] (15.2–17.8)
Main group II (HA-24), *n = 28*	3	8	9.3[1, 2] (8.6–10.3)	72.8[1-3] (70.9–73.9)	1.35[1] (1.17–1.42)	0.93[1] (0.85–0.98)	8.4[1, 2] (7.2–9.6)
	7	8	9.7[1-3] (9.0–10.7)	82.5[1-3] (80.2–83.8)	1.19[1] (1.03–1.33)	0.46 (0.34–0.56)	3.4[1] (2.0–4.4)
	14	6	10.4[1, 2] (9.5–11.2)	65.6[1-3] (64.2–66.5)	1.49[1-3] (1.46–1.56)	0.67[1, 2] (0.57–0.77)	6.7[1, 2] (5.2–7.8)
	28	6	11.0[1, 2] (10.3–11.6)	45.4[1-3] (44.0–46.7)	2.16[1-3] (2.08–2.17)	0.79[1-3] (0.70–0.88)	15.2[1-3] (13.9–16.5)
Main group III (HA-48), *n = 28*	3	8	8.3[1] (7.7–8.8)	80.1[1, 2] (77.7–81.5)	1.22[1] (1.18–1.32)	0.95[1] (0.89–1.03)	6.4[1] (5.2–7.5)
	7	8	7.9[1] (7.2–8.7)	86.1[1] (84.4–88.3)	1.14[1] (1.02–1.26)	0.41 (0.34–0.49)	2.8[1] (1.6–4.3)
	14	6	9.2[1] (8.3–9.9)	74.5[1, 2] (72.7–78.0)	1.32[1] (1.24–1.39)	0.53 (0.44–0.62)	4.5[1] (3.4–6.1)
	28	6	9.9[1] (9.1–10.4)	57.1[1, 2] (55.3–58.0)	1.72[1, 2] (1.71–1.74)	0.57 (0.48–0.65)	8.4[1, 2] (7.2–9.7)
Control group (without local injection), *n = 28*	3	8	7.4[1] (6.7–8.0)	86.5[1] (84.6–87.6)	1.11[1] (0.95–1.15)	0.97[1] (0.89–1.06)	6.0[1] (5.1–6.9)
	7	8	6.8[1] (6.3–7.8)	89.7[1] (87.4–91.0)	1.07[1] (0.91–1.21)	0.36 (0.27–0.44)	2.1[1] (0.8–3.2)
	14	6	8.5[1] (7.7–9.2)	81.4[1] (80.0–82.3)	1.20[1] (1.09–1.23)	0.35 (0.26–0.45)	2.6[1] (1.1–3.7)
	28	6	8.8[1] (7.9–9.5)	69.1[1] (67.4–70.5)	1.42[1] (1.39–1.47)	0.34 (0.28–0.48)	3.7[1] (2.3–4.9)

[1, 2, 3, 4] $p < 0.05$ – differences with parameters in animals of intact, control, HA-48, and HA-3 groups (here and in Table 2).

Table 2

Dynamics of myoglobin and potassium levels in the blood serum of rats after local injection of hyaluronic acid in different modes with experimental crush injury *Me* (Q_{25}-Q_{75})

Experimental groups	Observation period after injury, days	*n*	Myoglobin, ng / ml	Potassium, mmol / l
Intact animals *n=10*		10	77.5 (69.0–90.0)	4.1 (3.9–4.4)
Main group II (HA-3/24), *n* = 28	3	8	780.4[1, 2] (731.8–829.0)	4.8[1, 2] (4.5–5.4)
	7	8	647.5[1-3] (583.0–660.7)	4.6 (4.2–4.9)
	14	6	409.0[1, 2] (357.1–461.1)	3.6 (3.1–4.0)
	28	6	322.4[1] (272.2–374.4)	3.6 (3.3–4.4)
Main group I (HA-3/24/48), *n* = 28	3	8	786.2[1, 2, 3] (740.9–831.2)	4.9[1, 2] (4.7–5.3)
	7	8	634.2[1, 2] (586.8–679.1)	4.5 (4.0–4.8)
	14	6	411.4[1, 2] (368.9–453.0)	3.6 (3.3–3.8)
	28	6	318.1[1] (267.3–370.3)	3.6 (3.3–4.2)
Main group III (HA-3), *n* = 28	3	8	776.1[1, 2] (716.9–838.3)	5.0[1, 2] (4.7–5.6)
	7	8	694.0[1, 2, 3] (630.4–707.3)	4.1 (3.5–4.4)
	14	6	447.2[1, 2] (386.0–507.2)	3.5 (3.3–3.9)
	28	6	354.6[1] (303.9–406.4)	4.4 (4.1–5.0)
Main group IV (HA-24) *n* = 28	3	8	794.9[1, 2] (741.2–848.4)	5.8[1, 2] (5.5–6.4)
	7	8	768.2[1, 2] (721.2–815.7)	4.5 (3.9–4.8)
	14	6	549.3[1, 2] (497.9–601.6)	3.6 (3.3–3.8)
	28	6	346.5[1] (295.7–398.3)	3.8 (3.5–4.4)
Main group V (HA-48), *n* = 28	3	8	912.6[1] (861.9–964.7)	5.8[1, 2] (5.5–6.4)
	7	8	804.1[1] (757.1–851.6)	4.5 (3.9–4.8)
	14	6	584.5[1] (542.0–626.1)	3.4 (3.2–3.8)
	28	6	340.0[1] (289.2–392.2)	3.8 (3.5–4.4)

Table 2 (continued)

Experimental groups	Observation period after injury, days	*n*	Myoglobin, ng / ml	Potassium, mmol / l
Control group (without local injection), *n* = 28	3	8	971.0[1] (959.5–1,000.5)	7.6[1] (7.3–8.2)
	7	8	890.5[1] (832.50–960.0)	4.8 (4.2–5.1)
	14	6	701.0[1] (636.0–758.0)	3.6 (3.1–4.0)
	28	6	363.5[1] (318.0–409.0)	3.1 (2.8–3.9)

Thus, local administration of HA early after the cessation of compression (3 hours) in CI led to improvement in microcirculation in the injured area, increase in oxygen consumption, and activation of metabolic processes in tissues, which reduced the severity of destructive processes in them. The best effects were observed in the group of animals, which were injected with HA in the area of compression twice (3 and 24 hours after the cessation of compression).

DISCUSSION

The analysis of the obtained results showed that pronounced microcirculation and metabolism disorders with the predominance of the anaerobic oxidation pathway developed in the soft tissues of the compression area in CI. In response to local injection of HA, improvement in microcirculation in the damaged soft tissues was observed, as evidenced by the increase in the K_v coefficient by 32.4–72.1% ($p < 0.05$) compared with the control group. At the same time, circulation in the injured area had the highest values after local injection of HA after 3 and 24 hours in the early stages (3–7 days) after the cessation of compression.

The restoration of impaired microcirculation contributed to an increase in tissue oxygen saturation, which was reflected in the dynamics of SO_2 and *U* parameters. Thus, after local application of HA, regardless of the dosage regimen, a decrease in SO_2 was observed at all periods of the observation, which indicated an increase in tissue oxygen consumption against the background of circulation restoration. The greatest decrease in SO_2 values was observed in the animals that were injected with HA after 3 and 24 hours. Significant changes were revealed when assessing the *U* index, which tended to be higher when there was an increase in tissue oxygen consumption.

Local injection of HA led to an increase in *U* index mainly at a later time (days 14–28), which may be due to the fact that oxygen was required for intensive repair of soft tissues. Early single or two-fold (after 3 hours and additionally 24 hours) injection of HA contributed to an increase in tissue oxygen consumption not only on days 14–28, but also by day 3 of the observation. The injection of HA at a later time (24 or 48 h) after the injury led to an increase in tissue oxygen uptake only by the end of the observation period (day 28).

The average values of OCR and EOM after local injection of HA, reflecting the intensity of oxidative metabolism in compressed soft tissues corresponded to the microcirculatory status and oxygen consumption parameters. The most significant positive effect of HA was observed in the groups with its early administration (3 and 24 hours after the cessation of compression): in the HA-3 and HA-24 groups on days 14–28, in the HA-3/24 group – on days 7–28. Late injection of HA (after 48 hours) did not significantly affect the intensity of metabolic processes in the tissues of the injured area. The dynamics of EOM had a clear dependence on the mode of HA injection. Thus, local injection of HA in the HA-48 group contributed to an increase in its values by day 28, in the HA-24 group – by days14–28, and in the HA-3, HA3/24, and HA-3/24/48 groups – throughout the observation period compared with the values in control group.

In case of soft tissue damage, therapeutic measures are primarily aimed at replacing the tissue defect and activating regeneration mechanisms, which are possible only with the restoration of intercellular interactions [8]. HA in the injured area, possessing hydrophilicity and high biocompatibility, provides conditions for diffusion of nutrients and oxygen and migration of cells of the immune system [9].

The positive effects of HA in the early post-injury period are associated with facilitating the migration of immune cells capable of limiting the necrotic zone and disposing of destroyed cellular structures from tissues. The study showed that the most pronounced effect of HA was manifested with its early local administration (after 3 hours), while late injection (after 48 hours) was ineffective. This can be explained by the physiological mechanisms of restoration of injured tissues, which are activated immediately after the injury. These processes are genetically determined and proceed under the control of neurohumoral mechanisms [10].

In the early periods after the termination of compression in CI, local defense systems are activated in the tissues of the injured area in response to massive se-

cretion of inflammatory mediators, which is necessary to localize the lesion, eliminate the factors that caused it, and remove decay products. All this contributes to the preservation of the metabolic activity of cells in necrobiosis and triggers regeneration. Therefore, the biological action of HA is implemented at both cellular and intercellular levels. The cellular effects of HA are due to its ability to activate the receptors of cell membranes and change ion fluxes; intercellular effects are aimed at maintaining tissue homeostasis by forming a biochemically stable intercellular environment. In this regard, it is obvious that local injection of HA in the injured area in CI is ineffective when it is carried out later (48 hours after the injury). At the same time, single (after 3 hours) and multiple early (after 3 hours and 24 hours) local injection of HA is pathogenetically substantiated and has a positive effect on the formation of sanogenesis in CI.

CONCLUSION

1. Early local injection of HA in the injured area 3 hours after the cessation of compression improves microcirculation, increases oxygen consumption by tissues, positively affects the metabolism in the skeletal muscles, and reduces the severity of destructive processes in them.

2. The most effective method for correcting CI is early repeated injection of HA in the compression area 3 and 24 hours after the injury.

3. In case of CI, a single or multiple intramuscular injection of HA in the injured area in the early post-compression period is a sanogenetically substantiated method for correcting traumatic ischemia of muscle.

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Received 10.12.2021;
approved after peer review 14.02.2022;
accepted 10.03.2022