Reactive and destructive reactions of dorsal root ganglion neurons in the simulation of deep skin wounds healing at different rates

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Abstract

In an experiment on 216 male white rats, the changes occurring in the neurons of the dorsal root ganglion (DRG) for different models of healing the deep wounds of the skinwere studied. Naturally healing wounds with purulent complicated process, as well as the healing of aseptic and purulent wounds in the application of platelet concentrate (PC) were simulated as an experimental treatment factor. A connection between the severity of the manifestations of morphological changes in neurons and the rate of skin wound healing wasestablished. The least pronounced changes correspond to aseptic wounds in the application of the PC; the most prominent correspond to purulent wounds with the introduction of the PC. Differences in the reactions of A-and B-populations of sensory DRG neurons were also observed.

Key words: dorsal root ganglion, A- and B-type of neurons, deep skin wounds, platelet concentrate.

Реактивные и деструктивные реакции нейронов спинномозговых узлов при моделировании глубоких ран кожи с различной скоростью заживления

В эксперименте на 216 самцах белых крыс были изучены изменения, происходящие в нейронах спинномозговых ганглиев при различных моделях исцеления глубоких ран кожи. Были смоделированы естественно заживающие раны осложненные гнойным процессом, а также заживление асептических и гнойных ран при применении тромбоцитарной массы, как экспериментального фактора лечения. Установлена связь между тяжестью проявления морфологических изменений в нейронах и скоростью заживления ран кожи. Наименьшие изменения соответствуют асептическим ранам, наиболее выраженные соответствуют гнойным ранам. Наблюдались также различия в реакции чувствительных нейронов спиномозговых узлов А-и В-популяций.

Ключевые слова: узел заднего корешка, нейроны типов А и В, глубокие раны, кожа, тромбоциты, тромбоцитарная масса.

Studying the role of the afferent part of the nervous system in the process of regeneration can contribute to a better understanding of mechanisms of neurogenic regulation in this process with the purpose of developing methods to control it, in particular, ways of stimulating the regeneration process [6, 9].

For the identification of disease process in a case of chirurgical pathology, it becomes important to study the dysfunction of regulating mechanisms [4] since sensory neurons are one of the essential components of the autoregulation mechanism. Nowadays, a study of neurons of dorsal root ganglions (DRG) with the help of innovated methods for expediting the healing process of skin wounds, as the result of direct links between their morphofunctional state and the speed of reinnervation of the injured region, represents a certain interest.

The purpose of our research is to study changes in neurons of the dorsal root ganglions of the lumbar segments L_{III}-L_{V} during a process of healing with different rates of repair. We used the following models of the wound healing process:

1. Healing of conditional aseptic wounds (AW).
2. Healing of wounds complicated with purulent process (PW).
3. Healing of conditional aseptic and purulent wounds with the use of platelet concentrate (PC) as an experimental factor that changes the speed of healing process [1, 5] (AW + PC, PW + PC).

Leaving the detalization of clinical aspects of the healing process and mechanisms of the functioning of platelet concentrate, it is important to note that the use of the latter for the healing of aseptic wounds provided the fastest rates of healing.

The second fastest healing was attributed to natural healing in conditional aseptic wounds. The phase of active inflammation took place during the first 1-2 days, the phase of proliferation ended at around the 5th day, after which the processes occurring in the wound were directed towards reorganization of cicatrix. The purulent wounds demonstrated significant difficulty in healing and a decline in the speed of skin reparation. The model of the most complicated wound process represented the purulent wounds treated with platelet concentrate. That process showed the prolongation of the all stages of healing by 1-3 days.

Material and methods

The experiment was conducted by the Department of Normal Human Anatomy and the Institute of Chirurgical Infections of Voronezh State Medical Academy N. N. Burdenko. The study was done on 216 male adult white outbred rats weighing 250-280 gm. In pathogen-free conditions, we made an incision of 1 cm in length and 0.5 cm in depth on an anterior surface of the left thigh. The animals were organized into 5 groups: one group for the intact control and 4 experimental groups. In the first experimental group (conditional aseptic wounds), the process of wound healing had a conditional course place. Into the wounds of the second group of animals we applied platelet concentrate with concentration of thrombocytes not less than 10^6/μl. To modulate the purulent process, we deposited a culture of Staphillococcus aureus in the formed wound recess in a dose of 10^{10} microbes in 1 ml of 0,9% solution of sodium chloride. In 3 days after the incorporation of microbes, the platelet concentrate was applied once into the wounds of some animals.

In order to take experimental material out from the rats, we narcotized them by ester and performed decapitation. The animals were derived from the experiment on the 1st, 3d, 5th, 7th, 14th, and 28th days by equal groups of 7 in each, including the group of the intact control. We performed the incision of the lumbar ganglions L_{III}-L_V as they correspond to the neurons that innervate the wound region. A taken material was fixed in Karnua solution, embedded in histomix and cut in 6 μm sections. The sections ware stained in cresyl violet acetate.

On the light-optical level we studied the following characteristics of the neurons: the area of the profile field of the neuron, the area of the nucleus, and calculated the nuclear-cytoplasmic ratio. To determine the area of profile field of the nucleus and neuron, we performed digital photomicrography and processed obtained images using a graphics tablet and the program ImageJ ver. 1.68. Acquired figures of the area in pixels translated into μm^2 with the help of an integrated program and precalibrated converter.

While studying the neurons of dorsal root ganglions, we chose cells with morphofunctional characteristics of different functional states: the neurons that do not have noticeable changes, the neurons with reactive or reversible changes, and the neurons with destructive changes.

The estimation and comparison of a relative number of cells of described groups in 6 fields of vision were performed for each DRG. The characteristics of the reactive changes of the neurons were considered as the following: the presence of perinuclear chromatolysis, general hyper- and hypochromic cytoplasm, cases of shrinkage of the cells, and pericellular edema. The cells that had apparent pycnosis of the body, shrinkage of the nucleus with possible exit of the nucleolus, vacuolization, extreme hyper- and hypochromatism with impossible identification of basic cellular components were considered to be destructively changed neurons [7, 9].

Based on literature data [8] and analysis of the results of morphometrical studies, which showed the bimodel distribution of morphometric parameters of neurons of DRG, we identified two basic groups of the neurons: A-cells with an average diameter of 30 μm and a light perikarion as well as condensed distribution of Nissl substance; B-cells with an average diameter less than or equal to 30 μm, round cells with dark perikarion and diffused distribution of Nissl substance.

Statistical analysis of the obtained results was performed using a software package Statistica 6.0. The statistical analysis of the samples of absolute values of studied parameters showed the deviation from the normal distribution law. As a result, the comparison in separate groups was performed using the Mann-Whitney test; the results were considered reliable at $p < 0.05$. The comparison of relative values was conducted using the Fisher method.

The results of the study

The undertaken study demonstrated that in DRG of the control group, a major part of neurocytes were represented by cells without any patterns of reactive changes. Each neuron had one core of round shape with well-marked nucleolus. Nissl substance was heterogeneous: some neurons had large clumps of Nissl substance in perinuclear cytoplasm; in the other part of neurons, tigroid substance was dispersed throughout the cytoplasm. In DRG of the control animals, neurons with reactive changes, in general, constituted 6.4%, which according to literature data can be considered as a manifestation of normal functional polymorphism of cells.

The changes in DRG on the 1st day after making the incision were characterized by the appearance of primary irritation of neurons. It was observed that the number of cells with chromatolysis and expanded pericellular space increased.

On the 3rd day of the experiment in the neurons of DRG, the processes of chromatolysis increased, which were in a form of an expansion in the lightened region between the nucleus and tigroid substance which was displaced to the periphery of cytoplasm.

The total quantity of cells with the features of reactive changes constituted 26.7 ± 1.2%, while within histological sections of DRG, morphologically changed cells often formed isolated groups, out of boundaries in which resided unchanged neurons (fig. 1).

Similar changes in the neurons of DRG were found on the 5th day of the experiment. In one week after making the incision, a portion of the neurocytes with reactive changes increased. Among such neurons in large numbers we found cells with a condensed chromatophilic substance under the cellular membrane and with ectopia of the nucleus, which can be considered to be features of axonal reaction [2, 3].

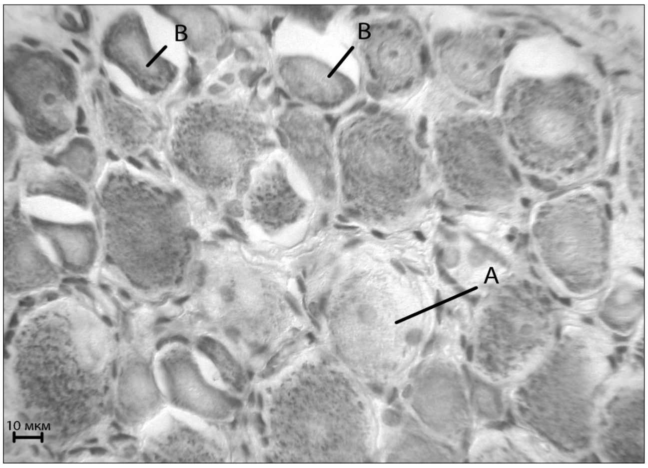

Fig. 1. Reactive changes in DRGneurons, 5th day of experiment, aseptic wounds. A – cell with chromatolysis, B – cells with piknosis. Cresyi violet. X 320.

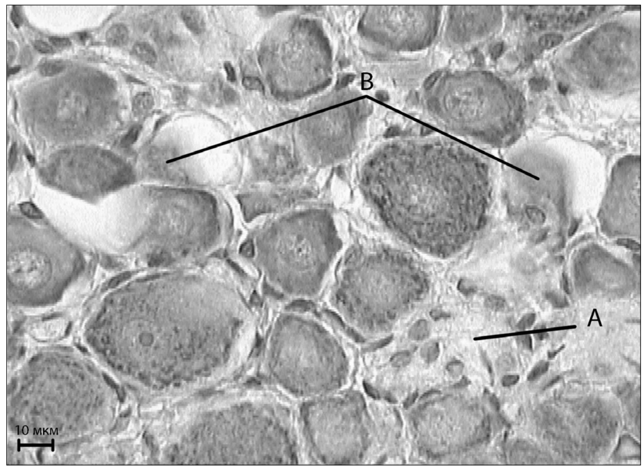

Fig. 2. Destructive reactions in DRG neurons14th day after injury. A – glial node, B – neurons with destructive reactions. Cresyi violet. X 320.

The impact of group of posttraumatic factors on this stage led to the partial elimination of neurons and initiated the processes of destruction, as a result of which the formation of regions with destructively changed cells took place. On the 14th day after the incision, neuronal reaction was expressed more strongly, and unequal and blurred edges as well as shrinkage with the formation of wide perineuronal cavities were noticed in large number of cells. In that period, we often observed cells with total chromotolysis and poorly noticeable boarders of nuclei. As the result of irreversible changes that led to death of neurons, sole glial nodules were formed [2, 3], as consequence of neuronophagia and following the migration of satellite glia (fig.2).

In 28 days, saved neurons of DRG came back to a normal state and the condensed form of the chromatophilic substance in the cytoplasm was restored. The number of cells with reactive changes decreased; however, the number of cells with destructive changes remained on the same level.

Studying the changes in neurons of model animals with different speeds of healing showed that, with the general direction of preserving the morphological changes, the magnitude and dynamics were significantly different. In close relationships of reactively changed cells at the end of 1stday of wound process, on the 3rd and 5th day of the experiment the number of cells with reactive changes in platelet concentrate wounds increased, the proportion ($p < 0.05$) of which were higher than those in the animals with a naturally wound healing process. From the 7th till the 28th day, the number of reactively changed cells in platelet concentrate wounds were lower than those in the control group (fig. 3).

It is particularly interesting that the number of cells with destructive changes in aseptic wounds, where the use of platelet concentrate was responsible for lowering the number of such cells, was more obvious at 28th day (fig. 4). The purulent process was characterized by a higher portion of cells with various deviations with the same dynamics as natural wound healing.

Studying the dynamic in the balance of A- and B-cells with different forms of reversible reactions showed that it depends on the specifics of the healing process, which complies with the large number of cells with destructive changes in prolonged healing (fig. 5, 6). It is observed that reactive changes in large numbers primarily occur in B-cells than in big A-cells. The highest numbers of reactive neurons are observed in DRG for wounds complicated with purulent process, and the lowest number is seen in clean wounds treated with platelet concentrate.

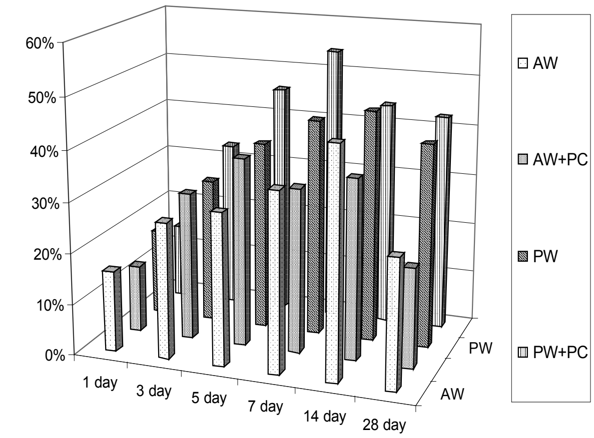

Fig. 3. Proportion of reactive changed neurons.

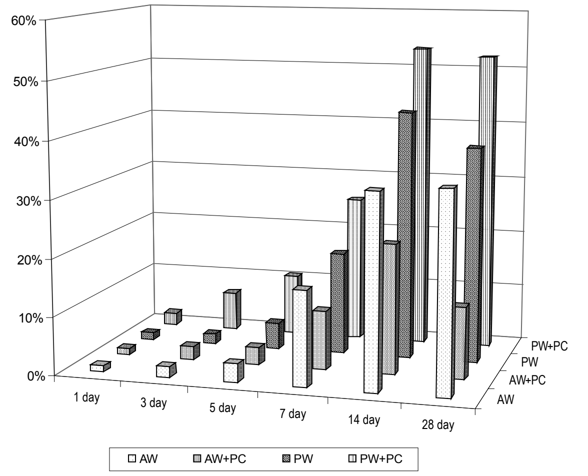

Fig. 4. Proportion of neurons with destructive reactions.

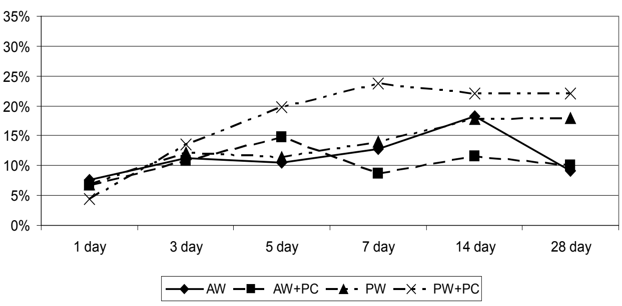

Fig. 5. Dynamics of the relative amount of A-type neurons with reactive changes.

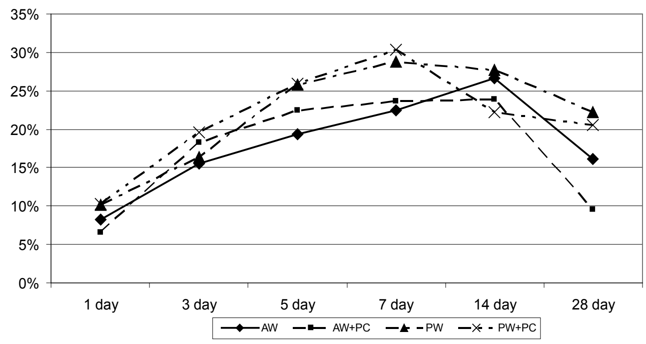

Fig. 6. Dynamics of the relative amount of B-type neurons with reactive changes.

The analysis of the dynamics in correlation of the studied groups of neurons differentiating themselves on the basis of area parameters shows that they restructured during the whole duration of the experiment. In the beginning period (up to and including the 3rd day), a decline in the relative numbers of B-cells took place, which then changed with constant increase up to and including the last day of the experiment. The A-cells demonstrated an opposite dynamic. It was noted that there is a link between dynamic changes and experimental factors: the use of platelet concentrate for clean wounds decreased the significance of restructurisations in the initial period of experiment, yet the presence of purulent process increase the range in correlation among the groups of the cells proportionally to the severity of wound process. The changes in the correlation of various cell groups were due to the following processes: increase or decrease of the area of the studied cells and onset of irreversible destructive changes in different times of the experiment, which led to the elimination of cells of different sizes [18].

The wound process not only impacted tinctorial qualities of the neurons, but also changed their morphometric parameters. Moreover, relative changes in volume of A- and B-cells occurred not only with different expression, but also at distinct times of wound healing. More apparent manifestation of B-cells were observed on the 5th day, while the main peak of A-cells' changes occurred on the 14th day. In this case, the measurements were done in the left neurons of DRG, and as a result of the sizes of cells uncomplicated inflammation were less different from those of the control. Yet in groups with aseptic wounds, marked changes in sizes reflected the state of reactively altered cells.

Conclusion

The neurons of DRG react to deep skin wounds with the complexity of compensatory and adaptive reactions, expressed in the formation of cell populations with various reactive and destructive changes.

The alterations in sizes of cells of saved neurons of DRG and their N/C ratio point at complex morphofunctional changes accompany various stages of wound healing. It is noted that there are differences in the reactions of A- and B-cells to the lesion: more intensive reactive changes in B-cells and the increase of them at the beginning of the research, while less intensive reversible processes in A-cells with the decrease of their number in late period of the study in comparison to the control.

The presence of purulent process in wounds and its complication causes more apparent changes in neurons of DRG throughout the duration of the experiment. The greatest speed of recovery demonstrates the model of conventional aseptic wounds with the use of platelet concentrate.

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Corpi adipoşi periaortici şi pericardiaci: aspecte actuale

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The periaortic and pericardiac fat pads: actual aspects

T. Hacina

This article contains controversial opinions from a literature review about the location, morphology, and functional importance of the periaortic and pericardiac fat pads, which has increased the interest for clinicians in recent years. The author's research results concerning these aspects are presented.

Key words: ascending aorta, periaortic fat pad, cardiac fat pad, atrial fibrillation.

Периаортальные и околосердечные жировые тельца: актуальные аспекты

Статья содержит анализ противоречивых данных, имеющихся в клинической и морфологической литературе о жировых тельцах аорты, сердца и перикарда, которые в настоящее время приобрели особую актуальность для специалистов торакальной хирургии. Приводятся данные собственных исследований по теме.

Ключевые слова: восходящая аорта, периаортальные жировые тельца, околосердечные жировые тельца, фибрилляция предсердий.

Actualitatea temei

Cea mai elocventă dovadă a actualităţii temei abordate o constituie lista impresionantă a articolelor referitoare la corpii adipoşi periaortali, epicardiali şi cardiaci, publicate în ultimul deceniu de către clinicieni, cât şi studiile morfologice relativ modeste şi, totodată, controversate în, care se elucidează această problemă.