Non-Classical Effects of the cAMP Accumulation Activators In Vivo

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Abstract

Purpose: Wound-healing dipyridamole- and papaverine-based aerosols (D1/D2) as activators of the accumulation of cyclic adenosine monophosphate are promising drugs that can accelerate wound healing in wound processes of various origins.

Methods: 128 rats were used in the study, including 38 in a pharmacological experiment on a model of stencil wounds and 90 in an experiment that studied the effect of spray on the number of CD34 cells in the blood of rats with chemically induced immunodeficiency. Immunodeficiency was caused by the fivefold administration of cyclophosphamide and prednisone. The expression level of CD34 was determined using flow cytofluorimeter.

Results: Dipyridamole- and papaverine-based aerosols of two compositions (with and without ascorbic acid) have pronounced reparative properties, significantly accelerating epithelialization and healing of stencil wounds in rats. In terms of this type of action, they are somewhat superior to dexpanthenol. Dipyridamole- and papaverine-based aerosols have the ability to produce beneficial effect on the entire body’s immune system by stimulating the division of pluripotent CD34 cells. The combined effect of papaverine and dipyridamole on tissues leads to selective stimulation of the division of pluripotent cells in the wound, and contributes to a six-fold acceleration of restoration of the animal’s immune system after induced immunodeficiency.

Conclusion: Topical application of D1/D2 aerosol samples on the skin of rats contributed to a statistically significant acceleration of regeneration processes. In terms of the appearance of granulations and epithelialization of wounds, D1/D2 aerosols were superior to dexpanthenol ointment.

Introduction

One of the main problems in the effective treatment of severe complications of type 2 diabetes mellitus is diabetic foot syndrome. Over time, the loss of sensitivity of the lower limbs at the late stages of diabetic neuropathy leads to a foot injury and the appearance of diabetic ulcers. These ulcers do not heal for a very long time; they become contaminated by bacteria, and in the end, would require surgical intervention.1 As of today, there is no effective remedy that could heal such trophic wounds. Experiments proved the ability of endogenous stem cells to significantly accelerate the wound healing process, particularly in diabetic patients (rat model).2 Another study has demonstrated that an excessive amount of cAMP in a wound can stimulate the migration of stem cells to the wound area and their rapid differentiation.3,4

As an agent for the treatment of trophic ulcers, we have developed a pharmaceutical composition in the form of an aerosol based on a combination of dipyridamole, papaverine and ascorbic acid. Papaverine and dipyridamole are universal phosphodiesterase inhibitors5,6 and inducers of cAMP accumulation. Ascorbic acid represents an indirect adenylate cyclase activator, and also, an inducer of cAMP accumulation and an important component of the tissue regeneration process in wounds.6 Using an animal model of Charcot-Marie-Tooth human disorder, was showed that ascorbic acid represses PMP22 gene expression by acting on intracellular CAMP concentrations. In this work, authors present kinetics data on the inhibitory effect of ascorbic acid upon adenylate cyclase activity.7

cAMP and cGMP are intracellular second messengers involved in the transduction of various physiologic stimuli and regulation of multiple physiological processes, including vascular resistance, cardiac output, visceral motility, immune response, inflammation, neuroplasticity, vision, and reproduction. Intracellular levels of these cyclic nucleotide second messengers are regulated predominantly by the complex superfamily of cyclic nucleotide phosphodiesterase (PDE) enzymes. Cyclic nucleotide PDEs comprise a superfamily of metallophospho hydrolases that specifically cleave the 3’, 5’-cyclic phosphate moiety of cAMP and/or cGMP.
Aerosol solutions were injected into a mechanical
wound healing

Determining the influence of D1 and D2 aerosols on stencil
(Kyivmedpreparat, Ukraine) and prednisolone (Darnytsia,
sodium ascorbate).

and for the second version of the aerosol (D2) it is 0.25%
hydrochloride (D1, 0.05% chlorhexidine as a preservative),

of active ingredients (0.1% dipyridamole, 0.1% papaverine
Ukraine); 10% polyethylene oxide-400; distilled water up
to 100%.

polyethylene oxide-400 (Medisca, USA); distilled water
papaverine and dipyridamole in combination with
the combination of two phosphodiesterase inhibitors,
has antiviral properties against a broad spectrum of
herpes viruses, probably through its immunomodulating
interferonogenic effect.

Papaverine also has a number of additional
immunotrophic properties that have not been previously
used in pharmacological practice. For example, papaverine
has antiviral properties against a broad spectrum of
viruses, due to both its direct antiviral effect and its ability
to stimulate the production of endogenous interferons.17-19

The goal of this study is to determine the ability of
the combination of two phosphodiesterase inhibitors,
papaverine and dipyridamole in combination with
ascorbic acid, to accelerate the healing of uninfected
wounds in an experiment.

Materials and Methods

Materials

The compositions were prepared on the basis of
dipyridamole (Medisca, USA) and papaverine (Aldrich/
Sigma, USA) in two compositions:

D1 (without ascorbic acid): 1% dipyridamole, 1%
papaverine hydrochloride; 0.05% chlorhexidine; 10%
polyethylene oxide-400 (Medisca, USA); distilled water
up to 100%.

D2 (with ascorbic acid): 1% dipyridamole, 1%
papaverine hydrochloride; 0.05% chlorhexidine (Viola,
Ukraine); 5 % ascorbic acid (LLC “Pilot Plant” GNCLS ,
Ukraine); 10% polyethylene oxide-400; distilled water up
to 100%.

Both aerosol variants are equivalent in terms of the ratio
of active ingredients (0.1% dipyridamole, 0.1% papaverine
hydrochloride (D1, 0.05% chlorhexidine as a preservative),
and for the second version of the aerosol (D2) it is 0.25%
sodium ascorbate).

In experiments also were used Cyclophosphan
(Kyivmedpreparat, Ukraine) and prednisolone (Darnytsia,
Ukraine)

Methods

Determining the influence of D1 and D2 aerosols on stencil
wound healing

Aerosol solutions were injected into a mechanical
nebulizer, and pressurized balloons were not used. To
enhance the penetration of active substances into the
affected tissues, 10% polyethylene oxide-400 was used as an
excipient in both versions of the aerosol20 Similar solutions
were also prepared for intraperitoneal administration,
but without chlorhexidine and polyethylene oxide-400. The concentration of ascorbic acid in the D2 variant was
reduced to 0.25%.

The wound healing action was studied on the basis of a
model of stencil wounds in rats. Their effect on the number
of animals’ own stem cells, CD34, was also evaluated.

The study of the wound healing properties of experimental aerosols D1/D2 was conducted on 38 Wistar
male rats.

The experiments involved Wistar male rats weighing
160–200 g. The animals were obtained from the
Biomodelservice (Kiev, Ukraine) and kept in vivarium in
Mechnikov Institute of microbiology and immunology.

The animals were divided into four groups: (1) wounds
treated with D1 aerosol (n = 10); (2) wounds treated with
D2 (n = 10); (3) wounds treated with the reference drug
dexpanthenol (Panthenol-Ratiopharm, 5% ointment) (n =
10); (4) control group (n = 8). Dexpanthenol is a vitamin
B5 - well-known substance in the world for tissues
regeneration stimulating.3,22

Stencil wounds23 of the back were simulated in animals
under anesthesia with diethyl ether. The initial wound size
was 4 ± 1.0 cm². The application of drugs was initiated
from the first day after the alteration. The average
amount of aerosol was 0.5 mL/animal. Applications were
performed twice a day to make sure that the drug covers
the entire surface of the wound and approximately 0.5 cm
of depilated skin around it. Then, the animals were fixed
for 30 minutes to ensure the absorption of the solution
and to prevent them from licking wounds. A planimetric
study24 was conducted on the 3rd, 7th, 10th, 14th and 18th
day from the beginning of the experiment. The obtained
parameters of the wound surface area were statistically
processed using the analysis of variance.

Stimulating the growth of CD34 + pluripotent
hematopoietic cells

The drugs were studied in an experiment on a model of
cytostatic hemoimmunosuppression.

The experiments were conducted on 90 male Wistar
rats weighing 160–200 g. The animals were kept
under conditions of free access to food and water. Two
experimental and one reference groups consisted of 30
animals each. In both groups, hemoimmunosuppression
was caused by a five-fold intraperitoneal administration,
at 24-hour intervals, of cyclophosphan (Kyivmedpreparat,
Ukraine) in a dose of 10 mg/kg, and prednisolone
(Darnytsia, Ukraine) 2 mg/kg in 3 ml of normal saline.
On the next day after the completion of the administration
of hemoimmunosuppressive drugs, the animals were
intraperitoneally injected with a D1/D2 composition in
cAMP activators in vivo as the wound healing agent

Table 1. The effect of D1/D2 aerosols on the surface area of stencil wounds in rats

| Drug product                  | Wound surface area, cm² (M±m) |
|------------------------------|-------------------------------|
|                              | Day 1 | Day 3 | Day 5 | Day 7 | Day 10 | Day 14 | Day 18 |
| D1(n=10)                     | 4.36±0.74** | 3.31±0.71** | 2.58±0.79** | 0.72±0.23* | -     |
| D2(n=10)                     | 4.21±1.06** | 3.40±0.87** | 2.12±0.73** | 0.78±0.70* | -     |
| Dexamethasone (n=10)         | 4.47±0.13** | 4.29±0.30** | 3.98±0.44** | 1.66±0.32* | -     |
| Wound control (n=8)          | 4.18±0.56 | 4.04±0.50 | 3.88±0.52 | 2.55±0.38 | 0.62±0.22 |

* P ≤ 0.05 (dispersions differ significantly versus the wound control parameters for the corresponding period).
** P > 0.1 (dispersions do not differ versus the wound control parameters for the corresponding period).

Starting from day 7, the differences between D1/D2 and the dexamethasone group were statistically significant at P ≤ 0.05.
eight rats still had an inflammatory process, and the area of the wound surface was large. Statistically significant differences between the experimental groups (including dexpanthenol group) and the reference group were noted as early as on day 7. During the same period, as well as on days 10 and 14, significant advantages of the experimental sprays vis-à-vis the action of dexpanthenol were observed. By day 18, wounds were completely healed in all rats of the experimental groups, while in the reference group, three animals did not complete wound epithelialization and one of them had pus discharge.

There were no statistically significant differences between the D1 and D2 groups. Therefore, ascorbic acid had no pronounced influence on the wound healing effect produced by the spray with dipyridamole and papaverine. Dexpanthenol ointment also helped reduce the wound healing time. However, its effect was somewhat less pronounced than the effect from the use of aerosols.

The administration of cytostatic drugs led to a three- to fourfold decrease in the level of CD34 by the time of the start of therapy comparing to the baseline level. Dipyridamole with papaverine in both compositions had a stimulating effect on the processes of bone marrow regeneration and restoration of peripheral blood parameters, increasing the level of CD34 to normal values. By day 30, its level increased more than twofold thanks to the effect from D1 and D2 samples, while in the animals from the reference group, it returned to normal values only on the 60th day. Therefore, dipyridamole with papaverine caused about a twofold acceleration of these processes.

The maximum effect from the use of the compositions was observed on the 60th day, and the restoration of the physiological level of pluripotent cells was observed even on the 10th day after the start of their use. The increase in the number of pluripotent cells coincided in time with the stimulation of reparative processes (on the model of stencil wounds in rats), which apparently indicates the predominant stimulation of stem cell division at the periphery rather than the enhancement of bone marrow functions.

The presence of ascorbic acid in the compositions had no bearing upon their effect on stimulation of the production of CD34 cells.

**Conclusion**

1. Dipyridamole- and papaverine-based aerosols of two compositions (with and without ascorbic acid) have pronounced reparative properties, significantly accelerating epithelialization and healing of stencil wounds in rats. In terms of this type of action, they are somewhat superior to dexpanthenol.
2. Dipyridamole- and papaverine-based aerosols have the ability to produce beneficial effect on the entire body’s immune system by stimulating the division of pluripotent CD34 cells.
3. The combined effect of papaverine and dipyridamole on tissues leads to selective stimulation of the division of pluripotent cells in the wound, and contributes to a six-fold acceleration of restoration of the animal’s immune system after induced immunodeficiency.

4. The absence of a statistically significant difference between the groups of D1 and D2 compositions indicates an insignificant role played in wound regeneration by ascorbic acid in the aforementioned dosage.

**Ethical Issues**

The animals were cared for according to the international guidelines GOST 33647-2015 (Principles of the Good Laboratory Practice, GLP), the international recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (The European Convention, 1986). The protocol of the experimental study was approved by the Ethics Committee of Mechnikov Institute of Microbiology and Immunology of National Academy of Medical Sciences (protocol № 3-2018 of 14.12.2018).

**Conflict of Interest**

Authors declare no conflict of interest in this study.

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