

DRUG DEVELOPMENT

Study on the Healing Effect of Syringaresinol β -D-Monoglucoside

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Abstract

The present study focused on the impact of lignane syringaresinol β -D-monoglucoside (substance **1**) on the healing process of the linear and planar wounds in white male Wistar rats, with a body mass ranging between 180 and 200g. This substance was found to contribute towards quicker healing of the wound damage, when compared with solcoseryl. Thus, the solidity of the cicatrix with the substance **1** injection exceeded that which resulted from the solcoseryl injection. Substance **1** was also found to exert an anti-inflammatory effect. However, in the intensity and timing of inflammatory stage changes it was analogous to the solcoseryl impact.

Keywords: wounds; healing; syringaresinol β -D-monoglucoside; solcoseryl.

Introduction

Plant tissues and cell cultures are widely used today in the pharmaceutical industry as alternative sources of biologically active substances¹. In a number of cases, the cultivated cells are found to contain substances, which are absent (or present in significantly smaller quantities), in the whole plant [1].

We extracted lignane syringaresinol β -D-monoglucoside (substance **1**) from the cell culture of *Scorzonera hispanica* L. [2] and characterized it. Previously [3] and later [4], this substance was identified in the plant *Cressa cretica* L [4]. Lignane **1** was found to be able to restore the functions of the cytotoxin-suppressed B- and T- immunity systems [5].

The present article sums up the data on the wound-healing impact of this substance acquired through the experiments with white rats. Lignane **1** increases the speed and quality of healing the linear and planar wounds more effectively than solcoseryl.

Methods

Suspense culture of *Scorzonera hispanica* L. cells was incubated in saline medium MS with the addition of 0.4 g/l of thiamin, 0.1 g/l of pyridoxine, 20 g/l sucrose in the dark on the agitator at 26°C. The cultivar was sustained via 10-fold dilution by fresh medium every 7 days.

Syringaresinol β -D-monoglucoside was extracted from cultivated cells according to the method developed by the authors [3] and had a melting temperature of 177–179°C (from ethanol) and $[\alpha]_{546}^{25} -7^{\circ}$ (c 0.72 ethanol).

A linear 5-cm long wound was made as far as its own fascia on an area of depilated skin on the backs of white male Wistar rats, with body weight ranging between 180 and 200g. At even intervals, three stitches were applied, apposing the wound edges. The test group animals were injected intramuscularly with lignane **1** at a dose of 5 μ g/kg in normal saline. They were administered 0.2 ml once a day for 8 days.

Wound healing was observed in the rats without medicines, used as the control. At the end of the test, tensiometry of the wounds was performed. To identify the strength of the cicatrix, a device for evaluation of the elastic rubber module VN 5307 was used. The principle of the method

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may be summed up as follows: a 1-cm wide and 3-cm long portion of skin from the back was fixed with one end at the upper end with a special clip, and the lower end of the skin was attached to another clip with a platform for weights, the mass of which was gradually increased until the cicatrix was torn. A larger mass required to destroy the cicatrix proved its greater strength.

Animals of the second group were injected intramuscularly with solcoseryl as the preparation for comparison on a daily basis over 8 days, at a dose of 0.2 ml per animal. After wounding, the animals were maintained under daily observation.

The planar skin-muscular wound was abdominally reproduced under barbamyil anesthesia. On the depilated skin sections on the back, a 400 mm² piece of skin was cut and peeled. Frames to ensure equal initial wound size were stitched to the wound edges and the skin defects were left open during the whole duration of the experiment.

Wound swab prints were used in the cytological study. To prepare them, fat-free object plates were brought into contact by touching the wounds, following the removal of the liquid discharge using cotton wool or gauze. The prints of the adhering cells were taken on the glass. The dried prints were fixed in Nikiforov's mixture, and then dyed according to Romanovsky-Giemsa's method. Cytology samples were analyzed following M. P. Pokrovskaya's method modified by D. M. Sheinberg. The quantity of neutrophilic granulocytes was determined in the swabs: (+) – singular isolated neutrophilic granulocytes between 5 and 10 in number in the visual field; (++) – their small agglomerations; (+++) – their considerable agglomerations; (+++++) – their agglomerations across the whole preparation. The cytological image also incorporated an assessment of its phagocytic character.

During the regeneration phase, the material for cytological study was collected by first scraping the wound surface in line with M.F. Kamayev's method. Then the wound surface was scraped, layer-by-layer. The scraping thus acquired was spread over the fat-free glass in a fine layer, then dried, fixed and dyed according to Romanovsky-Giemsa's method. From pictures of the wound surfaces, the scrapes illustrate the development of the regenerative processes occurring in the wound.

Clinical observations were conducted from the 1st to 21st days after the skin-muscular wound application. During this time period we assessed the wound state, quality and maturity of the granulations, character of the wound discharge, state of the surrounding tissues and timeframe for epithelialization and healing.

The rate of healing of the wound damage was assessed through wound patterns taken on celluloid film, and further with weighing on the torsion balance and determination of the wound square area. Following determination of the planar wound size, the percentage of decrease in the wound area in 24 hours was estimated. The calculation was performed as per the formula:
$$\frac{(S - S_n) \times 100}{S \times t}$$

S – area at the previous measurement; *S_n* – area at the current measurement, *t* – number of days between the measurements

A histological study was performed on the 7th, 14th and 21st day after application of the skin-muscular wounds and the injection of substance 1 and solcoseryl. The animals were subjected to light ether anesthesia and decapitated. Tissue fragments with the wound were then cut out together with the adjacent undamaged skin and subcutaneous tissue. The skin pieces thus cut out were fixed in 10% solution of neutral formalin. Histological slices were then prepared from the pieces placed in celloidin-paraffin on a sledge microtome. Then the slices were dyed with hematoxylin-eosin, picro fuchsin in accordance with van Gieson's method.

The experiments were conducted based on the principles of humane treatment of animals as provided by the Guide for Care and Use of Laboratory Animals (ILAR publication, 1996, National Academy Press). The data acquired in the tests on the wound healing effect of the substances mentioned above were subjected to statistical processing comprising estimation of the value of the differences by Student's *t* criterion.

Results and Discussion

In the group of rats that received the lignane **1**, casting off the crust formed on the 1st day, post application of the linear wounds, occurred 1-2 days earlier than in the animals that received the solcoseryl and 3 days earlier than in the control (untreated) animals. The cicatrix formed on the area of application in the animals of the first group was more solid compared to that in the animals receiving solcoseryl and those in the control group (Table 1).

Table 1
Results of wound cicatrix tensiometry in rats, g (*M*±*m*, *n*=7)

Time interval	Lignane 1	Solcoseryl	Control
8 days	315.0 ± 28.4	280.0 ± 34.8	157.0 ± 14.3
<i>p</i> *	< 0.05	< 0.05	

* - *p* value vs control

The data drawn in Table 1 showed that the use of substance **1** contributed to the formation of the cicatrix, the strength of which exceeded that in rats of the control group by 2 times, and that in the solcoseryl treated group by 1.13 times.

The histological study of the wound defect showed that the wound in the animals of the 1st group was covered with more mature epithelium, undergirded by granulation tissue with mature fibroblasts, a fibrous structure of connective tissue and a few fibroblasts, while in the group treated with the solcoseryl injection the wound was covered with less mature newly formed epithelium of unequal thickness as strata of light round cells with wide cytoplasm undergirded by granulation tissue.

The data presented in Table 2 demonstrate that in the prints from the skin wound surface of the rats of all three groups under study, the largest number of neutrophilic leucocytes was observed at all times in the control animals. A considerably lower accumulation of leucocytes was characteristic of the wounds of the animals treated with substance **1** on the 3rd day of the tests, which, in contrast to the control, showed

abatement of the inflammatory reaction. Later, the amount of leucocytes decreased significantly.

As of the 3rd day of the tests, the leucocytes were substituted for macrophages whose numbers increased in the following days. Simultaneously, unlike the control and the group of animals treated with solcoseryl, the swabs of the 3rd day of the tests contained young fibroblasts and profibroblasts. They later transformed into mature fibroblasts.

Table 2

Impact of substance 1 on the cryptogram of "wound imprints" of planar skin-muscular wounds of white rats

Parameters	Groups of animals	Timeframes, days			
		3	7	14	21
Leucocytes (including destroyed ones)	Control	++++/++	++++/+++	+++/+	++/+
	Lignane 1	+++/>++	+++/>+	+/-	-
	Solcoseryl	++++/+++	+++/>+	+++/>-	-
Macrophages (phagocyte)	Control	-/-	+/>+	+/>+	+/>-
	Lignane 1	+++/>+	++++/+++	+++/>++	+++/>++
	Solcoseryl	+++/>+	+++/>+	+++/>+	+/>+
Fibroblasts	Control	-	+	+	++
	Lignane 1	++	+++	+++	+++
	Solcoseryl	+	++	+++	+++

Therefore, substance 1 on testing revealed anti-inflammatory properties and the characteristics of stimulating the wound regeneration processes.

Wound healing in control animal group

On the 7th day, the wound surface was covered with a crust, covering a thin layer of exudate. It contained polymorphous-nuclear leucocytes, macrophages and immature fibroblasts. Granulation tissue only appeared on the wound surface showing vertically located capillaries with wide clearances. Besides, it demonstrated a large number of polymorphous-nuclear leucocytes. At the same time, simultaneously with the emergence of the granulation tissue, the epithelium began to grow around the edges of the wound. On the 14th day, the wound looked smaller. The wound edges had actively epithelialized. On the 21st day of the test, no animal within the control group showed complete wound healing. The remaining wound area was filled with newly formed connective tissue. It contained horizontally oriented capillaries, with respect to the increased mature granulation tissue. At this time, the granulation tissue was largely dominated by fibrous structures consisting partly of collagen fibers and partly of elastic fibers.

Wound-healing impact of solcoseryl

On the 7th day of the comparison preparation, the wound surface was covered with a scab, beneath which a small amount of exudate, consisting largely of round mono-nuclear cells, was found. The granulation tissue seemed more mature than that in the control animals, at the same time. It revealed a large amount of fibroblasts, adhering bunches of collagen fibers, a multitude of blood capillaries. The epithelium "crept over" the wound edges across a large area. Proliferation of the

hair follicular epithelium was observed. On the 14th day of the test, the area of fibroblasts grew and showed saturation of the granulation tissue with collagen fibers. The epithelium began to grow from the wound edges making it look thickened. On the 21st day, two out of seven animals showed complete wound healing. The complete healing was accompanied by the filling of the wound surface with granulation tissue. The defect was epithelialized from the wound edges by the "creeping over" of a multi-layer flat epithelium, which revealed a malpighian layer, capable of further growth and due to the growing hair follicles in the epithelium.

Wound-healing impact of substance 1

On the 7th day, the wound surface healing did not differ much from the healing caused by the solcoseryl use. The wound surface was covered with a fine crust. Beneath the crust, the inflammatory changes appeared quite moderate. The granulation tissue was insufficiently mature, but already at that time, it showed fibroblasts located in the parallel fibrotic folds. The newly formed tissue layers had a large number of newly formed capillaries fostering quicker maturation of the granulation tissue.

On the 14th day, the wound surface was covered with a tender granulation tissue. The wound was actively healed. The granulation tissue next acquired a more wavy structure. The process of the granulation tissue maturing in its intensity did not differ from that in the animals receiving solcoseryl (Table 3).

Table 3

Dynamics of wound healing with the use of preparation 1, mm² (M±m, n=7)

Preparations	7th day	14th day	21st day
Lignane 1	20.6±1.2 *	8.8±0.7*	4.2±0.8*
Solcoseryl	21.4±1.7 *	9.4±0.5*	5.1±0.2*
Control	27.3±1.4	17.9±0.4	11.3±0.3

* - $p < 0.05$ vs control

On the 21st day of observation, 2 out of 7 rats showed complete wound healing. In the other cases, the skin defects were filled with mature granulation tissue. The newly formed epidermis was unequal in thickness. As the Table shows, the animals receiving preparation 1 demonstrated more intense skin wound healing than the control, at all times of the test, and when compared with the effect of the solcoseryl, wound pharmacotherapy did not differ significantly.

The dynamics of the wound size changed according to the timeframes, as well as the daily decrease in the wound area in white rats are presented in Tables 3, 4.

Table 4

Daily decrease of skin wound area in white rats with the use of preparation 1 (% , n=7)

Preparations	7-14 days	14-21 days
Lignane 1	8.1	7.4
Solcoseryl	8.0	6.5
Control	5.0	5.2

Conclusion

Therefore, the use of syringaresinol β -D-monoglucoside (**1**) contributes to quicker wound surface healing when compared with solcoseryl. Therefore, it was found that with the injection of substance **1** the cicatrix strength exceeded that produced with the injection of solcoseryl. Substance **1** was also found to possess anti-inflammatory properties. However, in the intensity and timing of the inflammation phases it had a similar impact as that induced by solcoseryl.

In conclusion, it was found that substance **1** stimulates the healing of wound damage (linear and planar skin-muscular wounds) and speeds up the regeneration processes.

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