საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 3, №3, 2009
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 3, no. 3, 2009

Pharmacochemistry

Burn Healing Compositions from Caucasian Species of Comfrey (*Symphytum* L.)

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ABSTRACT. In the present work an attempt has been made to investigate the pharmacological efficacy of composition (C) containing 2.5% of high-molecular fraction from the roots of Caucasian species of comfrey – *Symphytum asperum* and *S.caucasicum*, consisting of crude polysaccharides and novel biopolymer – poly[3-(3,4-dihydroxyphenyl)glyceric acid] (PDPGA) in comparison with 2.5% allantoin ointment (A) in *in vivo* experiment (skin burn model). Ointment (C) appeared to be approximately fourfold active than ointment (A) and twice active than combined ointment (CA) containing 1.25% of high-molecular fraction from comfrey roots and 1.25% of allantoin when treatment of animals began immediately after burn induction. Significantly lower ($P < 0.01$) values of burn area in (C)- than in (A)- and (CA)-treated animals were observed since day 2 till the end of the experiment. The obtained results allow to suggest that the burn healing effect of the investigated composition results from synergistic action of its constituents in the second phase of wound healing, the inflammatory response. Ointment containing high-molecular fraction from Caucasian species of comfrey may be recommended for treatment of second- and third-degree burns. *© 2009 Bull. Georg. Natl. Acad. Sci.*

Key words: *Symphytum, high-molecular fraction, allantoin, crude polysaccharides, burn healing.*

Introduction

Agents containing comfrey (*Symphytum* L.) extracts have long been used in folk medicine due to various pharmacological properties [1,2]. They raise the immunological status of an organism, render antioxidant, anti-inflammatory and wound-healing action, which is commonly associated with allantoin. On the other hand, the aforesaid comfrey extracts as well as commercially available preparations contain hepatotoxic pyrrolizidine alkaloids which strongly restrict the use of these extracts in modern medicine except for external application [2-5].

Profound study of the chemical composition of the Caucasian comfrey species - *Symphytum asperum* Lepech and *Symphytum caucasicum* Bieb. has been carried out at I.Kutateladze Institute of Pharmacochemistry. Neutral glucofructan and acidic arabinogalactan were shown to be the principal and minor constituents, respectively, of crude polysaccharides from *S. asperum* and *S.caucasicum* roots [6]. A novel water-soluble high-molecular compound - poly[3-(3,4-dihydroxyphenyl) glyceric acid] (**PDPGA**) was revealed both in comfrey roots and stems extracts [7-10]. This polymer possesses high antioxidant and anticomplementary activity and can be considered as a potential anti-inflammatory, vasoprotective and wound healing agent [9,11,12]. Previously we reported about significant antiexudative activity of *S.asperum* crude polysaccharides in acute inflammatory process, modeled in mice [13].

The aim of the present research was to study the burn healing activity of novel pyrrolizidine alkaloids- and allantoin-free medicinal preparation from Caucasian species of comfrey in comparison with 2.5 % allantoin ointment.

Materials and Methods

Test and reference preparations. Pyrrolizidine alkaloids- and allantoin-free medicinal form has been developed - the 2.5 % ointment of water-soluble fraction of comfrey roots containing crude polysaccharides and **PDPGA** (**C**). Composition of distilled monoglyceride and glycerine was chosen as an ointment base. Reference preparations - 2.5% allantoin ointment (**A**) and combined ointment **(CA)** (1.25% crude polysaccharides +1.25% allantoin) were prepared using the same ointment base as described in [14].

Animal pretreatment. White inbred male rats weighing 200-220 g were used in all experiments. Animals were maintained in a 12-h night/day rhythm in groups of 5 animals per cage under constant access to water and food. All procedures adhered to regulations related to animal use, and experiments were performed in accordance with the accepted principles [15]. 24 hours prior to the beginning of each experiment the animals were clipped and 4ð2 cm skin area was depilated on back and left flank under light ether anesthesia.

Skin burn model

Wound healing properties were studied using a slightly modified skin burn model [16]. Burns were induced on depilated skin area using a special device with the established temperature scale and contact electrical heater (2 sm^2 square copper plate). This device allows to obtain area and depth standardized skin burns. In our case the temperature of a contact plate was 150^0C, exposition time – 10 sec. In these conditions burn corresponds to IIIA-degree in accordance with the clinical classification of burns [17]. Burn induction was carried out under ether anesthesia.

Four groups of 6 animals were randomized and investigated: groups 1, 2 and 3 were treated with **(C)**, **(A)** and **(CA)** ointments, respectively, while group 4 was left untreated and served as treated/untreated control. Treatment of animals began immediately after burn induction. Wounds were treated with 0.2 ml of ointment per wound once a day. Animals were inspected daily for the estimation of general condition (behavioural reactions, appetite, body weight, survival rate).

Estimation of healing process.

Clinical supervision over wound healing process was carried out daily, up to full healing. Wounds were examined for the presence of contamination, exudation, scab formation, and the wound area was measured using a transparent grid template (with the area of a single cell 0.25 cm^2). Once a week wounds were photographed. Wound healing effect was estimated by the reduction of injured area in relation to initial and calculated under the formula:

$$\Delta = (S_{exp} / S_{in}) \times 100\ \%$$

S_{in} - initial wound area on day 1.
S_{exp} - wound area on day of measurment.

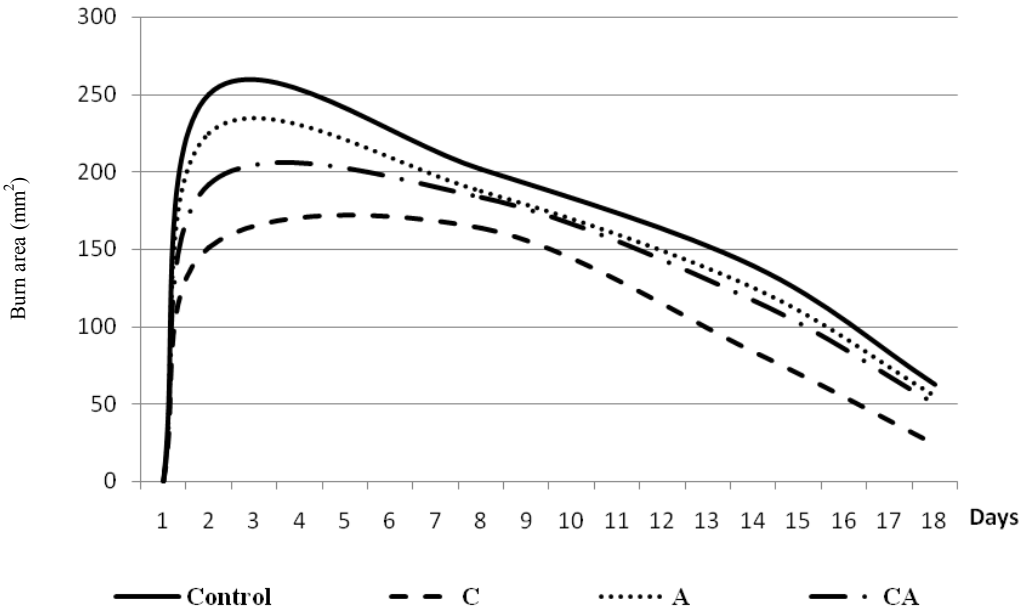

Fig. 1. Burn healing dynamics.

The obtained data were processed statistically using Student's *t*-test [18].

Results and Discussion

Burn wound area in animals, treated with ointments **(C)**, **(A)** and **(CA)** since day 2 of the experiment was, respectively, 40, 10 and 20% less than in control group ($p<0.01$) (Fig. 1). Primary eschars in groups **(C), (A)** and **(CA)** were torn away on day 8-10, while in control - on day 12-14. Full healing in animals treated with ointment **(C)** was achieved 3 days earlier than in **(A)** and **(CA)** treated animals, and 5 days earlier than in control group. No differences in healing dynamics were registered between control and ointment base treated animals. The results obtained revealed the expressed healing action of all ointments studied.

The common objective in wound management is to heal the wound in the shortest time possible. At the same time burn healing is complicated due to 1) extensive swelling initiated by the release of histamine and serotonin from platelets and mast cells and by kinins, which may interrupt blood flow, increasing the extent of injury as a result of anoxia and 2) incapacity of the burned tissue to produce timely an acceptable rate of fibrin at the locus of the wound [19].

Previously it was established that comfrey crude polysaccharides possess antiexudative activity [13], whereas **PDPGA**, in contrast to both allantoin and pure polysaccharides, exhibits marked antioxidant activity. Moreover, it was established that **PDPGA** inhibits the TNF-a production by human mononuclear cells/macrophages [9]. Taking all the above mentioned into consideration, it may be suggested that the burn healing effect of the investigated composition results from synergistic action of its constituents in the second phase of wound healing, the inflammatory response.

The established pharmacological action of ointment **(C)** allows recommending it for treatment of second- and third-degree burns.

Conclusions

1. Pharmacological study of burn wound healing activity of ointment containing high-molecular polysaccharide fractions from comfrey roots, free of allantoin and pyrrolyzidine alkaloids, revealed that by efficiency it is superior to allantoin ointment.

2. The obtained results allow to assume that established burn healing activity of the composition is associated with synergistic action of its constituents due to the shortening of the inflammatory phase of wound repair.

3. The established pharmacological action of ointment allows recommending it for treatment of second- and third-degree burns.

Acknowledgement

This work has been fulfilled by financial support of the Georgian National Science Foundation (Grant #GNSF/ST08/6-469).

ფარმაკოქიმია

დამწვრობის საწინააღმდეგო კომპოზიცია ლაშქარას (*Symphytum* L.) კავკასიური სახეობებიდან

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ნაშრომში მოცემულია ლაშქარას კავკასიური სახეობების *Symphytum asperum* და *S. caucasicum*-ის ფესვების პოლისაქარიდების ჯამის და ახალი ბიოპოლიმერის პოლი[3-(3,4-დიჰიდროქსიფენილ)გლიცერინის

მჟავას] (ᲐᲓᲒᲛ) შემცველი 2.5% მაღალმოლეკულური ფრაქციის შემცველი კომპოზიციის (ლ), 2.5%-იანი ალანტოინის მალამოს (ა) და კომბინირებული მალამოს (ლა) (რომელიც შეიცავს ლაშქარას 1.25% მაღალმოლეკულური ფრაქციას და 1.25% ალანტოინს), ფარმაკოლოგიური ეფექტურობის შედარებითი შესწავლა *in vivo* ექსპერიმენტში (კანის დამწვრობის მოდელი). მალამო (ლ), შესაბამისად, 4-ჯერ და 2-ჯერ უფრო აქტიური აღმოჩნდა, ვიდრე მალამო (ა) და კომბინირებული მალამო (ლა), როდესაც ცხოველების მკურნალობა დაიწყო დამწვრობის გამოწვევისთანავე. მალამო (ლ)-ით ნამკურნალებ ცხოველებში, დამწვრობის ფართობის სტატისტიკურად სარწმუნო შემცირება ($P < 0.01$) შეინიშნებოდა მალამო (ა)-სთან და (ლა)-სთან შედარებით ექსპერიმენტის მე-2 დღიდან ბოლომდე. მიღებული შედეგებიდან გამომდინარე, შესწავლილი მალამო (ლ)-ის სამკურნალო ეფექტი შეიძლება განპირობებული იყოს მისი შემადგენელი კომპონენტების სინერგისტული მოქმედებით დამწვრობის შეხორცების მეორე (ანთებით) ფაზაში. ლაშქარას კავკასიური სახეობების მაღალმოლეკულური ფრაქციის შემცველი მალამო შესაძლებელია რეკომენდებული იქნას მეორე და მესამე ხარისხის დამწვრობის სამკურნალოდ.

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Received April, 2009