

The use of hyaluronic acid derivatives in the treatment of long-term non-healing wounds

Systematic review and meta-analysis of RCSs (randomized controlled studies)

J. Voigt and V. R. Driver from Boston University School of Medicine (Boston, Massachusetts, USA) have conducted a systematic literature review and meta-analysis of randomized controlled study results (RCS), which confirmed the effectiveness of drugs based on hyaluronic acid (HA) in the treatment of long-term non-healing wounds, including: in diabetes mellitus (DM), neuropathic and chronic venous insufficiency (CVI). Compared to conventional therapeutic approaches and placebo treatment on the background of HA application these patient categories had either complete healing or significant reduction of wound defect sizes. Please find below the review of the article written by above-mentioned authors.

In the early 90th of the last century scientists had discovered the mechanism of HA binding to benzyl alcohol (esterification process), which allowed to use it in humans in such forms as tampons and films, without losing the unique properties and functions. Since then HA and its derivatives are used for the treatment of cutaneous and subcutaneous wounds of various aetiologies.

As HA is a hydrophilic compound, it can be considered as a biological lubricant. According to the RCSs the use of HA drugs for intraarticular injections (in the knee and ankle joints) in osteoarthritis, after arthroscopy, as well as in other lesions of the joints accompanied by sustained pain relief and improvement of function compared to anti-inflammatory therapy with corticosteroids, non-steroidal anti-inflammatory drugs and placebo (administration of saline solutions). Given that HA activates tissue hydrodynamics (including movement of cells), membranes based on it reduce the incidence and severity of adhesive processes in abdominal surgery.

HA is a polysaccharide, which is included in the connective, epithelial and neural tissues of the body. HA, involved in cell proliferation and migration, performs two important functions in wound healing. Firstly, it provides formation of temporary matrix in fresh wound, which promotes the diffusion of nutrients and wound cleansing from products of cellular metabolism. Secondly, HA is actively involved in the proliferation and migration of keratinocyte that make up the basic mass of the epidermis. Ultimately, this temporary structure is replaced with wound contraction by protein molecules - proteoglycans (providing tissue hydration and swelling pressure, preventing compression forces) and collagen. Since HA is hygroscopic macromolecule, it has a high osmolarity, which allows to control the hydration during phases of wound healing and related inflammatory reaction (when HA levels are increased). The presence of increased HA content during this process also has particular importance for cell proliferation and migration. Partially due to the presence of HA the cell fixation to the extracellular matrix is weakened, leading to detachment, facilitation of their migration and division.

HA degrades with the maturation of granulation tissue, an increasing number of protein molecules is produced under reduction of its level. Proteins bind to HA for transformation into proteoglycans, extension of wound healing process and increase of tissue elasticity. Water weight that is absorbed by HA molecule can exceed its own weight in 3 thousand times. Therefore, as previously mentioned, HA is an important tissue hydrating agent.

The purpose of this research was to study the outcomes of treatment of long-term non-healing wounds with drugs based on HA and its derivatives compared to other therapeutic methods or placebo.

Methods of the research

With the help of systematic reviews the authors of the research have attempted to summarize all the empirical evidence that match the given criteria in order to respond to a specific research question. Exact systematic methods we used in these reviews to minimize bias and obtain more reliable data. Systematic reviews may also include meta-analyses, which assumed the use of statistical methods to summarize the results of independent studies with similar outcomes. The following criteria were considered during selection of the studies for inclusion in the meta-analysis, such as their quality (RCSs with the highest level of quality); the presence of clearly formulated research question (for example, whether the HA drugs affect the wound healing); deciding what type of data is to be used: published or unpublished, and others.

Types of researches: prospective and randomized controlled studies to compare the effectiveness of drugs based on HA with other therapeutic measures and placebo.

Groups of participants: Patients with diabetic foot with the lesion down to the bone (Wagner grade 4), diabetic neuropathic ulcers and trophic ulcers of the lower limbs due to chronic venous insufficiency.

Prescribed drugs: tampons soaked in HA solution, gels and creams with HA; bandages, consisting entirely of HA (for example, hyalofill or hyalomatrix); membranes with HA used as a substrate for subsequent transplantation of autologous tissues.

Treatment outcomes: the primary endpoint — complete wound healing (defined as complete epithelialization of the affected area in the absence of purulent discharge); secondary — reduction of the area of wound defect.

Nine RCSs were identified during the search of the studies in the PubMed database, the Cochrane Library, on the websites of a number of authoritative scientific institutions and specialized journals. They met inclusion criteria for systematic review and meta-analysis, as well as given clinical endpoints (complete wound healing and reduction of the wound defect size in use of HA drugs compared to other therapeutic agents or placebo).

Results of the research

Detailed features of analysed RCSs to study treatment outcomes of long-term non-healing wounds of lower limbs using drugs based on HA and derivatives thereof (alone or in combination with other drugs) are presented in the table.

Table 1. Features of RCSs using drugs based on HA and its derivatives in the treatment of chronic wounds of the lower limbs of various aetiologies

Study	Participants	Curative measures	Outcomes	Wound aetiology
J. P. Ortonne, 1996	HA group: average age — 66.2 + 3.1 years; m/f -16/10; wound surface area at the beginning of the study - 20.8 ± 4.4 cm ² ; presence of the wound for at least 3 months; dextranomer group: average age - 69.7 ± 3,6 years; m/f - 17/7; wound surface area at the beginning of the study - 23.18 + 4, D cm ² ; presence of wound for at least 3 months. No difference was observed between the groups at the beginning of the study	After primary surgical treatment tampon soaked in HA was daily applied on the ulcer for 21 days (n = 26); wound was cleaned daily prior to application of the HA. Paste based on dextranomer (standard of care) was applied daily on the affected area (n = 24); wound was cleaned daily prior to dextranomer application	A statistically significant difference in the reduction of the wound surface area (cm ²) in favour of HA was established at the end of 21-day treatment (p <0.05, Mann-Whitney test). HA application was accompanied by a significant reduction in the occurrence and severity rates of oedema (p <0.001) compared to a slight similar effect in the standard therapy group A significant reduction in the incidence and severity degree was observed in the HA group on day 14 (p <0.001). Such effect in dextranomer group was not revealed until the 21st day of treatment (p <0.001)	Trophic ulcers of the lower limbs in CVI
M. Edmonds, A. Foster, 2000	Hyalofill bandage group: average age - 58 + 12 years; the average duration of the existence of ulcer - 45 ± 55 weeks; there were 13 ulcers with fistula and 13 with lesion to the bone in 15 enrolled patients . Control group: average age - 55 + 12 years; the average duration of the existence of ulcer - 48 ± 64 weeks; 9 ulcers with fistula and 9 with lesion to the bone were observed in 15 patients	Hyalofill bandages + standard therapy (primary debridement, removal of external pressure on the foot skin and infection control) (n = 15). Hyalofill bandage were applied at weekly intervals for 12 weeks, or until ulcer healing. Patients of the control group received only standard treatment (n = 15)	A statistically significant difference in favour of HA application group compared to the control group according to the frequency of wound healing after 12 weeks: ulcer healed in 10 of 15, in 3 of 15 patients of these groups, respectively (p <0.05)	Diabetic foot

J. R. Mekkes, M. Nahuys, 2001	In ten patients with long-term non-healing ulcers due to chronic venous insufficiency (n = 8) or vasculitis (n = 2) bandages with HA to one side of the wound and the intrasite gel bandages were randomly applied	Bandages with HA (n = 10), coated with a polyurethane film were applied once daily until 80-100% of the wound surface was not covered with granulation tissue. Then the wound defect was closed with graft. Intrasite gel bandages (n = 10), coated with a polyurethane film were applied once daily until 80-100% of the wound surface was not covered with granulation tissue. Then the defect wound was closed with graft	The time interval before transplantation decreased by 29% with HA bandage application (p = 0.004). The total time required for ulcer healing reduced by 31% when using HA (p = 0.0003)	Trophic ulcers of the lower limbs in CVI and vasculitis
C Caravaggi et al., 2003	Autologous tissue-grafts were placed in one group of patients on a composite membrane containing HA benzyl ester (HYAFF 11): the ulcer defect area - 5.3 ± 6.76 cm ² ; the presence of a wound within 4.0 ± 10 months; DM type 1/2 - 9/34; ABI - 0.73 ± 0.3; HbA _{1c} level - 7.9 ± 2.13%; Wagner grade 1-2. Paraffin gauzes were applied to the wound in the other group: the ulcer defect area - 6.2 ± 7.58 cm ² ; the presence of a wound for 4.0 ± 6 months; DM type 1/2 - 3/33; ABI - 0.7 ± 0.22; HbA _{1c} level - 8.1 ± 2.25%; Wagner grade 1-2. No difference was observed between the groups at the beginning of the study	Before treatment initiation all wounds were surgically treated. Autologous tissue-grafts were placed on the membrane HYAFF-11 and transferred to the wound (n = 43). Outcomes were assessed by complete healing of ulcers after 11 weeks. Paraffin gauze were used and additionally sterile gauze bandages were applied in the control group (n = 36).	No statistical difference in the rates of complete wound healing (in the plantar and dorsal surface of the foot) after 11 weeks of treatment (p = 0.191) was revealed between the groups. However, a statistically significant difference in complete healing of ulcers in favour of use of membranes HYAFF-11 was observed in the subgroup of patients with ulcers on the dorsal surface of the foot (p = 0.049). At the end of the study wound exudate was absent in 86% of patients vs. 69.4% of patients in HYAFF-11 and control group, respectively, with the significant difference in the healing of ulcers on the dorsal surface of the foot.	Diabetic ulcers on the dorsal and the plantar surfaces of the foot
L. Abbruzzese et al., 2009	The group of patients with HA applications (vulnamin gel): average age - 61.8 ± 8.9 years; DM type 1/2 - 2/13; duration of DM - 21.9 ± 6.7 years; HbA _{1c} level - 8.8 ± 1%; ABI - 1.1 ± 0.2; ulcer area - 25.8 ± 8.8 cm ² ; the presence of a wound for 30.8 ± 16.7 weeks. Inert gel (placebo) group: average age - 62, D ± 7.4 years; DM type 1/2 - 3/12; duration of DM - 19.8 ± 4.2 years; HbA _{1c} level 8.6 ± 1.2%; ABI - 1.0 ± 0.1; ulcer area - 27.3 ± 10.4 cm ² ; the presence of a wound for 22.9 ± 18.6 weeks. No difference was observed between the groups at the beginning of the study except the duration of the existence of ulcers	HA applications + elastic compression bandage application (n = 15) for 3 months or until ulcer scarring. Using inert gel (placebo) + elastic compression bandage application (control group) (n = 15) for 3 months or until the ulcer scarring	The surface of the ulcer defect decreased significantly in HA group after 4 weeks of treatment compared to the control group - 58.7 and - 23.4%, respectively; p < 0.05) Percentage of ulcer lesions, covered with granulation tissue after 4 weeks was significantly higher with HA application compared to the control group (62.8 ± 14.7 and 28.3 ± 10.2%, respectively; p < 0.01)	Neuropathic ulcers of the lower limbs
L. Uccioli et al., 2011	Autologous tissue-grafts were placed in one group of patients on a composite membrane containing HA benzyl ester (HYAFF 11): the ulcer defect area - 8.8 ± 9.4 cm ² ; the presence of a wound within 7.4 ± 6.6 months; DM type 1/2 - 11/68; ABI - 0.9 ± 0.2. Paraffin gauzes were applied to the wound in the other group: the ulcer defect area - 6.7 ± 7.7 cm ² ; the presence of a wound for 7.3 ± 7.8 months; DM type 1/2 - 6/74; ABI - 0.9 ± 0.7. NB! The area of the ulcer was larger in the group of HA applications (p = 0.016)	Autologous tissue-grafts were placed on membrane HYAFF-11 and transferred to the wound (n = 80). Outcomes were assessed by ulcers healing rate, their complete scarring in the 12th and 20th weeks, and after 18 months. Paraffin gauzes were used and additionally sterile gauze bandages were applied in the control group (n = 80).	The reduction of the ulcer area by 50% was achieved much faster in HA group (after 40 vs. 50 days on average, p = 0.018); ulcers on the dorsal surface of the foot healed significantly faster in patients in this subgroup (p = 0.047)	Diabetic foot

M/f - males/females; ABI - ankle-brachial index; HbA_{1c} - glycated haemoglobin.

Research on the effectiveness of HA applications compared to conventional therapeutic measures in patients with trophic ulcers of the lower limbs on the background of CVI

The authors studied the outcomes of the HA applications versus standard therapy in patients with trophic ulcers of the lower limbs in CVI in two RCSs. In one of them (Ortonne J. P., 1996) it was established that the percentage reduction in wound area after 3-8 weeks was significantly higher in the HA group compared to the standard treatment. In another study (Mekkes J. R., Nahuys M., 2001), bandages with HA to one side of the wound and the intrasite gel bandages were applied in patients. The time interval to the skin transplantation, as well as the time required for the ulcer healing was significantly reduced on the background of HA applications.

Research on the effectiveness of the HA applications and standard treatment in patients with diabetic ulcers on the dorsal and plantar surfaces of the foot (Wagner grade 1,2 or 4).

In two RCSs, researchers evaluated the feasibility of HA drug application in patients with diabetic foot. In the study of C. Caravaggi et al., (2003) in patients with diabetic foot ulcers Wagner grade 1-2 membranes based on HA with keratinocytes + subsequent transfer of autologous tissue-graft (transplantation was performed in about 7-10 days after HA application) were used. According to the obtained results, in a subgroup of patients with ulcers on the dorsal surface of the foot the healing occurred much faster compared to the standard treatment. In a more recent study by L. Uccioli et al. (2011) a similar category of patients was involved, however, the sample size and duration of observation were greater. After 20 weeks of treatment it was found that in a subgroup of patients with ulcers on the dorsal surface of the foot HA applications + keratinocytes followed by transfer of autologous tissue-graft compared to standard therapy (paraffin gauze application on the wound) had a significant effect on wound healing. In addition, the reduction of the ulcer defect area by 50% was achieved much faster in group of HA applications (after 40 vs. 50 days on average).

Studies of the effect of drugs based on HA on the healing of neuropathic ulcers of lower limbs compared to conventional therapy

In the study by M. Edmonds, A. Foster (2000), that involved patients with diabetic foot syndrome (Wagner grade 4 ulcers with lesions to

the bones), comparing the outcomes of standard therapy with HA applications it was found that complete wound healing after 12 weeks was observed in a greater number of cases in the HA application. In another RCS (Abbruzzese L. et al., 2009), scientists compared the efficacy of HA in the form of a gel + standard treatment versus the inert gel (placebo) + standard therapy. As a result a statistically significant effect was demonstrated on the size reduction of the ulcer after 4 weeks of treatment in patients of HA group.

According to the meta-analysis of data from these two RCSs (Edmonds M., Foster A., 2000.; Abbruzzese L. et al, 2009) the significantly higher ulcer healing rate and fewer amount of wound defects uncovered with granulation tissue compared to standard treatment was observed after 12 weeks after initiation of treatment in patients with neuropathic form of diabetic foot syndrome on the background of applications of HA derivatives.

Discussion of research results

According to the above-mentioned six studies, the efficacy of drugs based on HA has been proved in the treatment of chronic wounds of the lower limbs of different aetiology - complete healing or significant reduction in size of the wound defect, regardless of their form (solutions, gels, tampons, films, membranes).

It is interesting that the HA applications are effective in healing the most difficult currently treated long-term non-healing wounds, for example, in diabetic foot syndrome. This is a surprising finding given the fact that the pathogenesis of DM is associated with chronic inflammation. The initial formation of granulation tissue is an inflammatory process with a high degree of activity and intense tissue metabolism. HA facilitates this process, being present in large quantities in fresh granulation tissue. In other words, it is involved in the inflammatory process at the earliest possible stage. In addition, HA may also reduce inflammation in wound healing (Chen W. Y. J., Abatangelo G., 1999). When using derivatives of HA, their "modulating" effect on chronic inflammation that often occurs in DM is probably manifested. Thus, there is an acceleration of wound healing. In other diseases such as osteoarthritis, drugs based on HA have demonstrated anti-inflammatory and analgesic effect (Neustadt D., Altman R. D., 2007).

Regarding diabetic ulcers on the plantar surface of the foot and the lack of differences in outcomes between HA application group and control group in the study both by C. Caravaggi et al. (2003) and L. Uccioli et al. (2011) it should be noted that this type of diabetic ulcers can be

more sensitive to external pressure on the skin of the foot and the load on the wound (Spencer S. A., 2000; Wu S. C. et al., 2005). C. Caravaggi et al. came to the conclusion that foot unloading and not the use of drugs for their care has fundamental role for the healing of plantar ulcers in DM. This issue has also been studied in RCS by D. G. Armstrong et al. (2001), which compared the efficacy of the use of fixed (total contact casts), removable immobilization unloading bandages on shin and foot, as well as unloading low shoes in relieving of neuropathic foot ulcers in patients with DM. It has been found that the most effective reduction of the pressure on the foot and its maximum unloading were achieved using fixed immobilization unloading bandages. In their application for 12 weeks significantly more patients were cured compared to other methods of unloading of the foot ($p = 0.026$).

Another important aspect related to the healing of ulcers in diabetic foot syndrome is their size at the time of initiation of treatment. It is known that according to projections the wound with area of $> 5 \text{ cm}^2$ must scar in about 4 months (Margolis D. J. et al., 2000; Oyibo S. O. et al., 2001). The ulcer sizes exceeded this value in participants of the study by C. Caravaggi et al. (2003) and L. Uccioli et al. (2011).

The results showed that after 11 weeks of treatment no statistical difference was observed in the healing of wounds on the plantar and dorsal surfaces of the foot between the HA application group and standard therapy group. Therefore, taking into account the large sizes of ulcers in patients of both studies the outcomes of their treatment should be evaluated after a longer period of observation.

Conclusions

The RCSs described above confirm the efficacy of drugs based on HA and its derivatives in the treatment of long-term non-healing wounds of the lower limbs of various aetiologies compared to the conventional therapy. Further it is necessary to carry out longer clinical studies (> 12 weeks), particularly involving patients with wounds of larger sizes ($> 5 \text{ cm}^2$). It is also advisable to carry out RCSs on the effect of HA therapy on ulcers of arterial genesis.

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