Data concerning the cicatrization and regeneration action of a combination containing silver nanoparticles

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Abstract

An amount of 20 ppm of silver nanoparticles of less than 20 nm diameter were added to a comercially available antiseptic cicatrization powder and the effect of this addition on the healing properties of the mixed powder was investigated on deep skin lesions experimentally induced on rabbits. The plagues treated with the silver nanoparticles doped cicatrization powder showed an early formation of granular tissue, a faster contraction and scars of smaller area as compared to the wounds treated with the control powder. The microscopic analysis revealed a higher density of hair follicles in the transition area from the scar tissue to the normal tissue and a better epithelization, aspects indicating a higher quality of healing.

Keywords: cicatrizant powder, silver nanoparticles, healing

Introduction

Medicinal preparations and formulations containing silver nanoparticles are increasingly used in the treatment of skin lesions taking into account their antiseptic, antifungal and anti-inflammatory properties. These properties have been revealed by bacterial and fungal cultures during the research for developing a topical antimicrobial gel (1). Investigations on treatments of patients with burns have revealed that an ointment containing silver nanoparticles is superior to unguents containing silver ions in healing of superficial second-degree burns (2). Healing and regenerative actions of the composite materials with silver nanoparticles were evidenced by experiments on laboratory animals performed by our research team, considering the stimulation of hair follicles stem cells as possible mechanism of action (3, 4, 5, 6, 7). Based on these data we investigated in this paper the effect of adding silver nanoparticles on the healing properties of a commercially available antiseptic cicatrization powder used in treatment of deep rabbit skin experimental lesions.

Materials and Methods

The research has been conducted on six male rabbits aged 2, New Zealand race. Prior to experiments, the rabbits were anesthetized - epidural anesthesia with 0,2 ml/kg lidocaine 2% (8). Subsequently, the hair in the lumbar region of every animal was cut and full thickness experimental skin lesion were performed on each side of the vertebral column by excision under aseptic conditions, using plastic templates of 4 cm-2 surface. Throughout the experiment animals were given acetaminophen 37 mg/kg in drinking water (9, 10). Right side lesions were considered as control lesions and were treated after surgery only by application of Manis (commercially available healing and antiseptic powder of veterinary use, containing 5.50 g nitrofuran, 10 g Magnesium sulfuricum and 89.50 g Lactose). The lesions on the left side were treated with Manis homogeneously mixed with 20 ppm Ag nanoparticles of 20-30 nm size, proportion and size reported as optimal for healing and regenerative action of composite materials with silver nanoparticles (3, 4, 5, 6, 7). Wounds were covered with gauze dressing patched with OMNIFIX Elastic. Daily, the dressings were changed and the lesions were examined macroscopically. Throughout the experiment the rabbits have been clinically examined and the start of wound contraction, contraction rate and healing time were assessed. Daily, until day 7 and day 18 included, the wounds were photographed and the surface of each wound was estimated using the method described by Kıış et al (11). The wound images on the 7th were used to measure the areas occupied by the granulation tissue. Comparative statistical analysis of the experimental results was performed with ANOVA single factor test (significant results if $p \leq 0.05$). A lesion was considered healed when its entire area was covered with epithelium. After healing, on the 20th day after surgery, scars surfaces were determined and statistically compared by the same methods mentioned before. Skin fold thickness at the scar and normal skin from the same region was measured and the results statistically compared using single factor ANOVA test. At that time, under the protection of local anesthesia with novocaine, tissue fragments were collected that included scar tissue and normal tissue. Tissue fragments were fixed in neutral formalin concentration 15% and processed through inclusion in paraffin, cut in 4 μm sections using a microtome and then colored by hematoxylin-eozin method (HE) for optical microscopy analysis using Leica DM 5500Q TCS SPE microscope with DFC 290 camera.

Results

The first highly vascularized granular tissue areas were observed on the periphery of lesions treated with Manis powder mixed with silver nanoparticles. Wound contraction began in the 4th day. On the 7th day a faster wound contraction and the presence of a wider band of highly vascularized granulation tissue in the peripheral region of the left side wounds became macroscopically evident, as confirmed by statistical comparison of the concerned areas ($p \leq 0.05$) (Fig. 1, Table 1). Starting from the 8th day scabs formed on both groups of lesions, so that that healing process could not be assessed until their separation which started on day 14th. Scabs separation started first on left side lesions (Fig. 2) and was completed for all wounds on day 18, except for some small regions in the center of lesions that remained non-epithelialized (Fig. 3). The same as for day 7, on the 18th day the average surface of wounds on the left side was significantly lower than the average area of wounds on the right side. The contraction rate was higher for the first group of wounds (Table 1). On the day 20th all the wounds were completely healed (Fig. 4) and statistical comparison showed a significantly lower average size of scars on the right side as compared to those on the left side (Table 1). Despite that the analysis with ANOVA single factor test revealed a statistically insignificant correlation ($p>0.05$), the skin fold average thickness was higher for the scars on the right side (3.66 mm) as compared to the scars of the left side (3, 31 mm) and to the normal skin of the lumbar region (2.43 mm). Macroscopic observation revealed the presence of more intense pigmentation areas of the left side scars periphery, a phenomenon absent in case of the other group of scars (Fig. 3, 4).

Table 1

	7th day	18th day		7th day		20th day
Average surface of plagues on the left side (cm^2)	1,60 ± 0,01	0,03 ± 0,0002	Average surface of granular tissue on left side wounds (cm^2)	0,75± 0,01	Average surface of scars on the left side (cm^2)	0,42±0,0004
Average surface of plagues on the right side (cm^2)	1,77 ± 0,01	0,12 ± 0,001	Average surface of granular tissue on right side wounds (cm^2)	0,58± 0,01	Average surface of scars on the right side (cm^2)	0,5±0,001
	P = 0,04	P = 0,0001		P = 0,02		P = 0,001

Fig. 1. Lesion aspect on 7th day.

Fig. 2. Lesion aspect on 14th day.

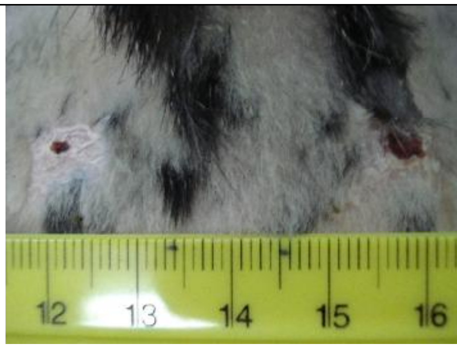

Fig. 3. Lesion aspect on 18th day.

Fig. 4. Complete healing on 20th day.

Microscopic analysis of transition areas from normal tissue to scar tissue revealed a greater number of hair follicles with a higher amount of melanin in case of scars resulted by treatment with Manis mixed with Ag nanoparticles as compared with the scars resulted after treatment with simple Manis (Figs. 5, 6). Moreover, the Manis mixed with Ag nanoparticles produced a better epithelization and a faster healing as demonstrated by the lower quantity of mature granulation tissue observed (Fig. 5). The wounds treated with simple Manis showed younger granulation tissue indicating a delayed healing with persistence of inflammatory infiltrate (Fig. 6).

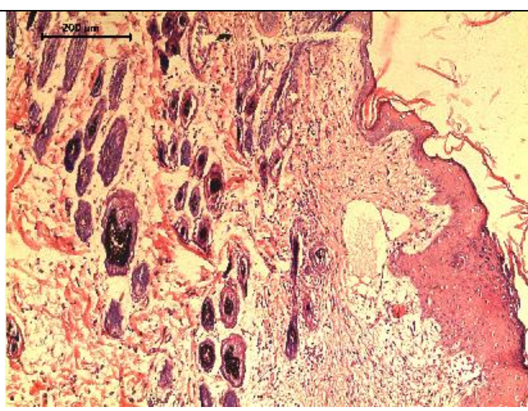

Fig. 5. The aspect of the transitory area between normal tissue and scar tissue in case of wounds treated with Manis

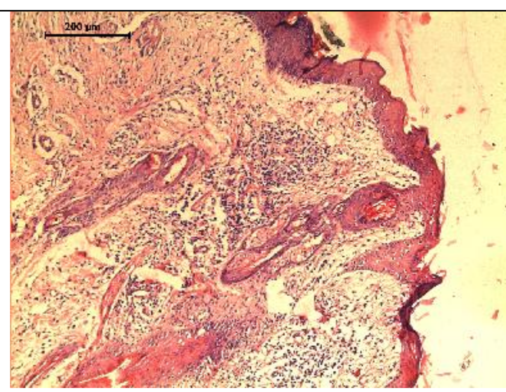

Fig. 6. The aspect of the transitory area between normal tissue and scar tissue in case of wounds treated with Manis. Histological

containing silver nanoparticles. Histological section (HE ob. 10x).	section (HE ob. 10x).

Discussions

Early formation of a well-vascularized granulation tissue may facilitate the acceptance of skin grafts (12), which recommends the Manis containing silver nanoparticles as a material useful in tissue engineering. Because the granular tissue has the characteristics of a contractile tissue due to its fibroblasts content (13), the more rapid contraction of the left side wounds was due to a larger amount of granular tissue located peripheral. The smaller thickness of the defects filled with connective tissue observed microscopically confirm the lower skin fold of scars resulted from wounds healed after treatment with Manis mixed with silver nanoparticles. This result, together with the observed hair follicles growth stimulation, proves functional and aesthetic benefits of Manis added with silver nanoparticles in treatment of deep skin lesions covered with hair, by comparison to the commercially available Manis. Stem cells in the hair follicles bulge, although usually do not play a role in the epidermis regeneration, are involved in healing acute skin lesions migrating through epidermis to the wound center, taking the phenotypic appearance of epidermal cells and after several weeks being removed (14). In mice, the multipotent stem cells contained in hair follicles can form in vivo all the cell lines of the skin covered with hair (new hair follicles, sebaceous glands, epidermis) (15) and in vitro these stem cells can differentiate into neurons, glial cells, smooth muscle and melanocytes (16). The stem cells in hair follicles can differentiate also into cells of the superficial dermis (17). Based on these facts it can be considered that the faster healing with early formation of a highly vascularized granular tissue, the microscopic evidence of a better epithelization and the presence of a greater number of hair follicles with increased melanin content in the lesions treated with silver nanoparticles containing Manis is due to a stimulation of hair follicles stem cell differentiation determined by the silver nanoparticles. This hypothesis is supported by the finding that the iontophoretically released silver stimulates tissue regeneration (18). In vitro studies suggest that at concentrations below 250 mg/ml silver nanoparticles exert an oxidative stress which may be countered by the antioxidant mechanisms of cells. Above this limit concentration silver nanoparticles induce cell apoptosis, which could produce wound healing without scars (1). Given these issues we believe that the observed hair follicles stem cells stimulation could be the consequence of a mild apoptotic action of silver nanoparticles, cell apoptosis which could contribute to high quality healing. The lower intensity inflammation observed on the microscopically investigated samples taken from lesions treated with Manis added with silver nanoparticles may be partly the result of antiseptic and anti-inflammatory actions of silver nanoparticles observed on bacteria cultures (1). By comparison with preparations containing silver sulfadiazine available on the market (1% silver sulfadiazine), the antibacterial activity of silver nanoparticles occurs at concentrations 30 times lower than the silver concentration in the antimicrobial gel formulated by Jain J. et al in 2009 (1), or about 25 times smaller than silver in gelatin nanofibers functionalized with silver nanoparticles at the same concentration like that used in the present work (19). One could consider that the cicatrization powder investigated here has the advantage of using a very small concentration of silver nanoparticles (which reduces the risk of toxicity), concentration at which the regenerative effect is maxim and still demonstrate antiseptic and anti-inflammatory effects. The risks of adverse effects occurring through inhalation of the powder is extremely low given that experimental studies on rats have shown that the nasal mucosa and lungs of animals exposed to 61 μg/m3 of silver nanoparticles in an inhalation room 6 hours per day, 5 days per week, 28 days, showed no significant toxicological effects (20), and can be further reduced by specific methods of protection.

Conclusions

1. Skin wounds treated with Manis containing silver nanoparticles showed early formation of granular tissue, faster contraction and lower average surface os scars as compared to wounds treated with commercially available Manis.
2. Microscopic analysis of scars resulting from application of Manis added with silver nanoparticles showed a higher density of hair follicles with increased melanin content at the periphery of the scar tissue, a better epithelization with lower amount of mature granulation tissue, almost no inflammatory infiltrate, all these indicating better and faster wound healing.
3. Stimulation of granulation tissue formation with strong vascularization may facilitate the acceptance of grafts, recommending the silver nanoparticles containing Manis powder as a material suited for tissue engineering, its healing and regenerative properties being useful especially in treating lesions of skin covered with hair.

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