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Original Article

Exogenous Nitric Oxide Plays Important Roles in Wound Healing and has an Antibacterial Effect

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ABSTRACT

Background and Aim: While air plasma has been widely used in biomedical or clinical applications, nitric oxide produced with plasma is thought to play important roles and be significant in biological activities. Our aim is to elucidate the role of exogenous nitric oxide in wound healing and antibacterial effect. **Methods**: 140 Wistar rats weighing 180 to 230 g were used. They were injured and irradiated by NO-containing plasma. **Results**: Wound healing was promoted in case of NO-containing plasma therapy. **Conclusion**: Exogenous nitric oxide prompts wound healing and has an antibacterial effect.

Keywords: Nitric oxide, Exogenous nitric oxide, Wound healing, Antibacterial effect, Plasma therapy, Experimental model.

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INTRODUCTION

Nitric oxide (NO) is an important messenger that regulates numerous physiological functions and also participates in the pathogenesis of various diseases.[1] NO is believed to play an important role in various aspects of skin physiology including wound healing and inflammation.[2] The double-edged or double-faced role of NO was described in some articles.[3,4] While many studies advocate the adequate usage of NO, especially exogenous NO, in physiological and clinical application,[5-7] others stressed the harmful effect of NO.[2,3,8] Exogenous NO inhibits the physiological activity of pathogen such as bacteria and virus.[6,7] In case of the insufficiency of endogenous NO (which is produced by the immune system to respond to infection and exert broad-spectrum antibacterial action[9,10]) for physiological causes, exogenous NO has been delivered as an inhaled gas to treat premature infants[11,12] and more recently, respiratory diseases.[13-16] Our research team has developed nitric oxide-releasing plasma jet and used exogenous NO as alternatives to conventional antibiotics. We explain how exogenous NO may influence histopathological changes in experimental models and can be used as an antibacterial agent.

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MATERIALS AND METHODS

The experiments were performed in the Pathology Research facility of Chongjin College of Medical Sciences.

Materials

140 Wistar rats weighing 180 to 230 g were used (regardless of sex). The Staphylococcus aureus strain was obtained from the Department of Microbiology, Chongjin College of Medical Sciences. NO-releasing plasma jet was purchased from Laboratory for developing medical appliance of Chongjin College of Medical Sciences.

Experiments were approved by local animal ethic committee.

Methods

After sterilized on rats' abdomen with 0.1% chlorhexidine hydrochloride, they were anesthetized with 5% ketamine hydrochloride intramuscularly (1 mg/kg).

After fixed in a fixed frame with their back up, their back hairs were shaved 3 cm by 3 cm on their backs. The full-thickness flap was excised at the size of the 2×2 cm until superficial fascia appeared. Staphylococcus aureus (10^9 CFU/mL) was floated with a platinum loop and spread evenly on the wound surface. Using NO-containing gas produced from plasma jet, the wounds of study group were irradiated.

Using Griess reaction, the nitric oxide content (C_{NO}) and nitrogen dioxide content (C_{NO2}) were analyzed. The content of NO was 700~900 ppm and the irradiation time was 90~120s in the experimental models. They were irradiated twice a day. Rifampicin capsule (China) was orally administered in control group (30mg/kg).

Bacterial colonization in wound secretion was counted as follows. Before wound treating, the secretions in the wound were picked with a platinum loop, spread evenly on an agar culture medium, and incubated at 37 °C for 24 hr, and the number of colonies was counted. Also, we evaluated the course of wound healing such as macroscopic and microscopic findings.

Statistical Analysis of Data

Data was plotted by SPSS 22.0 (IMB, Armonk, NY) as mean ± standard error of the mean. Student *t* test was used to check statistical significance where applicable. Differences were considered statistically significant for $p < 0.05$.

RESULTS

Macroscopic Findings of Wound

The size of wound area according to the treatment duration tended to be slightly smaller than before treatment in both groups on 3rd day of treatment, and then gradually decreased, with significant differences between the control and study groups from 7th days of treatment ($p < 0.05$) (Figure 1).

Microscopic Findings of Wound

We microscopically evaluated wounds. (Olympus, Japan). The capillaries were significantly higher in the study group compared to the control group on days 7 and 11 of treatment, and there was no significant difference on day 15 in both groups (Figures 2, A, B). The epithelialization in study group was significantly increased from 3rd day compared to the control group according to duration (Figures 2, C, D). The inflammatory cell infiltration was significantly reduced from day 11 of treatment compared to the control group in the study group (Figures 2, E, F).

Anti-bacterial Effect of Wound

We evaluated the colonies in secretion of wounds in both groups. The colonies in the wounds decreased rapidly compared to rifampicin group (control group) day by day (Table 1).

DISCUSSION

This study examined the histopathological change and antibacterial effect of exogenous NO. Many studies have been made regarding the histopathological findings and antibacterial

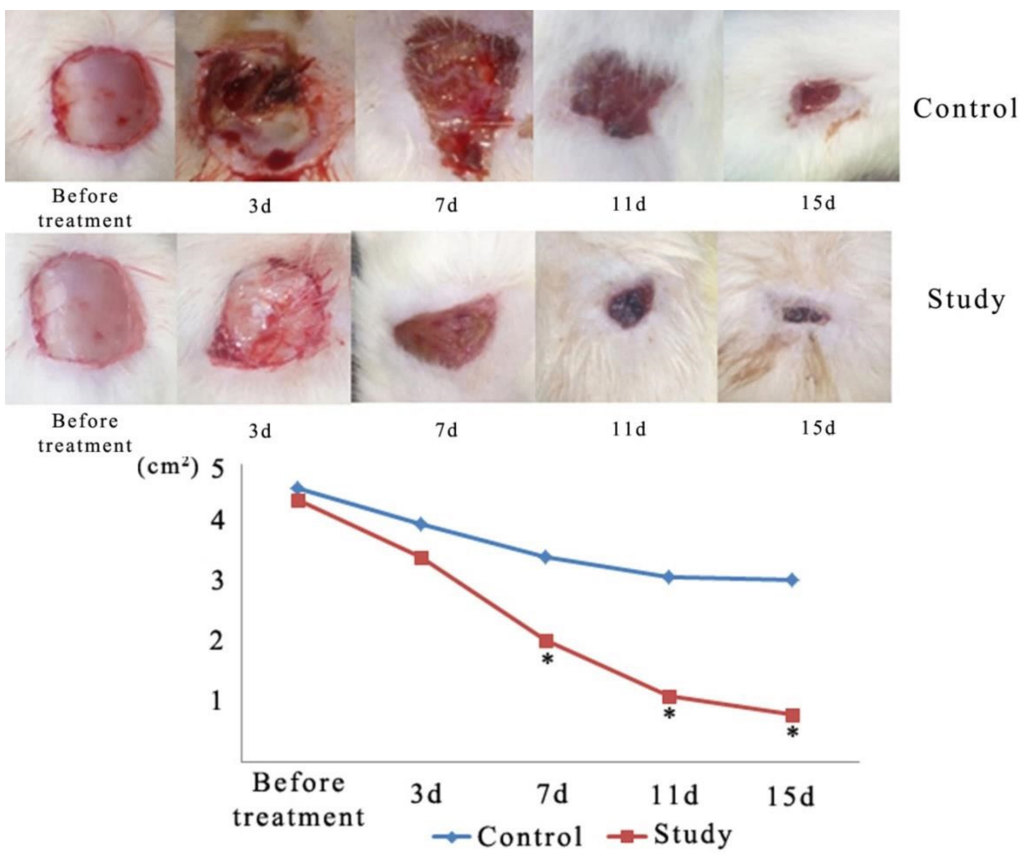

Figure 1: Macroscopic appearance of wounds according to treatment duration in the study and control groups.

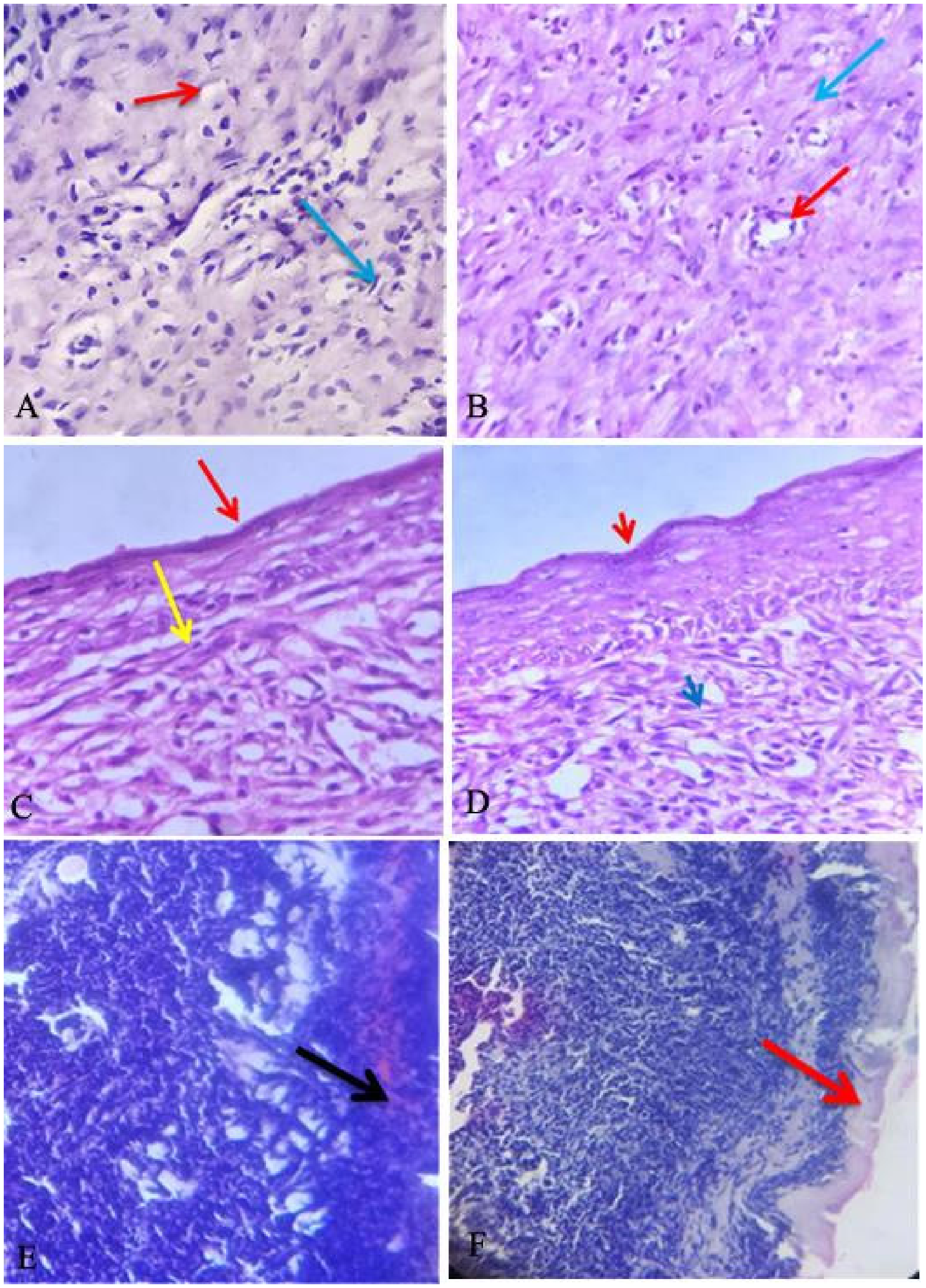

Figure 2: Microscopic findings in both groups. ×400, left: control group, right: study group. Every tissue sample was dyed with hematoxylin-eosin stain. (A) As the granulation tissue matures, fibroblasts gradually become collagen fibers and capillaries become venules. Red arrows indicate capillaries, blue arrows indicate collagen fibers. (B) As the granulation tissue matures, fibroblasts gradually become collagen fibers and capillaries become venules. Red arrows indicate capillaries, blue arrows indicate collagen fibers. (C) A thin epidermis layer was formed. Red arrows indicate epithelial tissue, yellow arrows indicate dermal connective tissue. (D) The epidermis was completely formed. Red arrows indicate epithelial tissue, blue arrows indicate subepithelial connective tissue. (E) There is a high infiltration of inflammatory cells in the necrotic exudates. Black arrows indicate exudates. (F) There is a high infiltration of inflammatory cells in the necrotic exudates. The red arrow indicates exudates.

effect of exogenous NO.[6,7,17,18] While many authors described the effect exogenous NO was dose-dependent,[17,18] others advocated the harmful effect of it [2,3,8]; our result shows the positive effect of NO in the sense of not only macroscopic and microscopic findings but antibacterial effect. Although we have not demonstrated a dose-response curve for exogenous NO, it seems, in a low content, exogenous NO plays a positive role in the wound healing and antibacterial effect. They described the wound healing effect as well as antibacterial effect of exogenous NO.[6,7,17,18] More studies should be required whether the more content of exogenous NO, the more negative effect has occurred. Anyhow, exogenous NO must have a very positive effect on wound healing and antibiotics

Table 1: CFU Changing in infectious wounds (n=7).

	Number of CFU	Treatment duration (day)			
		1	3	7	11
Control group	5<	0	0	1 (14.28)	2 (28.57)
	6~9	0	1 (14.28)	2 (28.57)	5 (71.43)
	10~14	2 (28.57)	3 (42.86)	4 (57.14)	0
	<15	5 (71.43)	3 (42.86)	0	0
Study group	5<	0	0	5 (71.43)	7 (100.0)
	6~9	0	4 (57.14)	2 (28.57)	0
	10~14	4 (57.14)	2 (28.57)	0	0
	<15	3 (42.86)	1 (14.28)	0	0

in appropriate doses. Studies on the dose-dependent effect of NO should be continued.

CONCLUSION

Exogenous nitric oxide significantly accelerates wound healing and reduces bacterial load in infected wounds. It enhances angiogenesis and epithelialization while decreasing inflammation. The antibacterial effect was superior to conventional antibiotic treatment. Appropriately dosed exogenous NO may serve as a promising alternative or adjunct in wound management.

CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

ACKNOWLEDGEMENT

None.

ABBREVIATIONS

NO: Nitric Oxide; **CFU:** Colony Forming Units; **CNO:** Concentration of Nitric Oxide; **CNO_2:** Concentration of Nitrogen Dioxide.

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