Effects of Different Compression Time Windows and Intervention Modalities on Skin Injury and Ischemia-Reperfusion in a Pressure Ulcer Rat Model: Postprint

Authors: Wang Huajun, Dai Shixue, Lu Quan, Linchang Ye, Li Hua, Song Xi, Hong Tao, Sha Weihong

Date: 2018-06-15T00:00:00+00:00

Abstract

Objective: To observe the effects of different compression time windows and intervention methods on skin injury and ischemia-reperfusion in a pressure ulcer rat model. Methods Sixty-eight SD rats were randomly divided into a blank group (Group S, n=4, no compression) and a model group (n=64); based on whether intervention measures were taken, the model group was randomly divided into two subgroups: no-treatment (Group a, n=32, sacrificed directly after compression) and postconditioning (Group b, n=32, subjected to ischemic postconditioning after compression before sacrifice), and skin compression degree, neutrophil infiltration degree, and serum oxygen free radical levels were observed after 2, 4, 6, and 8 hours of compression (n=8 for each time point). Results The difference in skin injury degree composition among Groups S, a, and b was statistically significant (P=0.001), with Group b being milder than Group a; regarding time windows, the 6-hour compression group had 2 rats with severe injury, and the 8-hour group had 6 rats with severe injury (37.5%); while no severe injury was caused by 2-hour or 4-hour compression, the difference in injury degree among different time windows was statistically significant (P=0.043); in terms of neutrophil infiltration degree, Group b had more rats with Grade I (mildest) than Group a (n=17vs n=15), and fewer with Grade II than Group a (n=8 vs n=10), the difference in infiltration degree under different intervention methods was statistically significant (P=0.002); the infiltration degree caused by 2-hour compression was the mildest, followed by 4-hour and 6-hour; 8-hour was the most severe, the difference in neutrophil infiltration degree caused by different compression times was statistically significant (P=0.027). Regarding ischemia-reperfusion, with prolonged intervention time, both Groups a and b showed decreased serum superoxide dismutase content and increased malondi-

aldehyde and nitric oxide, overall the reperfusion injury degree at 2-hour and 4-hour was lower than that at 6-hour and 8-hour; intergroup comparison showed that Group b' s superoxide dismutase content was significantly higher than Group a' s, while malondialdehyde and nitric oxide were lower than Group a' s (all P<0.05). Conclusion Ischemic postconditioning can alleviate acute pressure ulcer ischemia-reperfusion injury, its protective effective time window is within 6 hours of skeletal muscle ischemia, with the protective effect being better at 4 hours and optimal within 2 hours. Ischemic postconditioning can effectively ameliorate oxygen free radical damage and inflammatory response caused by skeletal muscle ischemia-reperfusion injury in rats.

Full Text

Preamble

Effect of Different Time Windows and Interventions on Skin Pressure Ulcers and Ischemia-Reperfusion Injury in Rats

WANG Huajun, DAI Shixue, LU Quan, YE Linchang, LI Hua, SONG Xi, HONG Tao, SHA Weihong
Department of Gastroenterology, Guangdong General Hospital and Guangdong Academy of Medical Sciences, Guangzhou 510080, China

Abstract

Objective To observe the effect of different compression time windows and intervention modalities on skin injury and ischemia-reperfusion in a rat pressure ulcer model. **Methods** Sixty-eight SD rats were randomly divided into a blank control group (Group S, n=4, no compression) and a model group (n=64). Based on whether intervention was administered, the model group was further randomized into two subgroups: a no-intervention group (Group a, n=32, sacrificed directly after compression) and a postconditioning group (Group b, n=32, received ischemic postconditioning after compression before sacrifice). Skin compression degree, neutrophil infiltration, and serum free radical levels were observed after 2, 4, 6, and 8 hours of compression (n=8 for each time point). **Results** Significant differences were found in skin damage severity among the three groups (S, a, and b) (P=0.001), with Group b showing milder injury than Group a. Regarding time windows, 2 rats exhibited severe damage after 6 hours of compression, and 6 rats (37.5%) showed severe damage after 8 hours, while no severe damage occurred after 2 or 4 hours. The difference in injury severity across time windows was statistically significant (P=0.043). For neutrophil infiltration, Group b had more rats with Grade I infiltration (mildest) than Group a (n=17 vs. n=15), and fewer with Grade II infiltration (n=8 vs. n=10), with the difference between intervention modalities being statistically significant (P=0.002). Compression for 2 hours caused the mildest infiltration, followed by 4 and 6 hours, with 8 hours causing the most severe infiltration (P=0.027). Regarding ischemia-reperfusion injury, both Groups a and b showed decreased serum superoxide dismutase (SOD) content and in-

creased malondialdehyde (MDA) and nitric oxide (NO) levels with prolonged intervention time, with overall lower reperfusion injury in the 2- and 4-hour groups compared to the 6- and 8-hour groups. Intergroup comparison revealed that Group b had significantly higher SOD content and lower MDA and NO levels than Group a (all $P<0.05$). **Conclusion** Ischemic postconditioning can alleviate ischemia-reperfusion injury in acute pressure ulcers. The effective time window for this protective effect is within 6 hours of skeletal muscle ischemia, with optimal protection achieved within 2 hours. Ischemic postconditioning effectively improves oxygen free radical damage and inflammatory responses induced by skeletal muscle ischemia-reperfusion injury.

Keywords: pressure ulcer; ischemia-reperfusion; ischemic postconditioning; time window; animal experiment

Introduction

Pressure ulcers are localized injuries to the skin and/or underlying tissue resulting from sustained pressure, particularly over bony prominences and/or accompanied by shear forces, which cause local circulatory impairment and ischemic damage. Their formation results from the combined action of multiple internal and external factors. Research has shown that ischemic preconditioning and postconditioning are effective measures for mobilizing endogenous protective mechanisms, enhancing tissue and organ tolerance to ischemia-reperfusion injury. Remote postconditioning of distant tissues can also reduce damage to the primary ischemic tissue. Zhao et al. confirmed in a canine myocardial ischemia-reperfusion injury model that ischemic postconditioning could reduce myocardial infarct size, protect endothelial function, and decrease neutrophil aggregation. Ischemic postconditioning (I-postC), implemented after the onset of reperfusion, has been successfully applied in experimental and clinical studies of reperfusion injury in skeletal muscle, brain, intestine, spinal cord, kidney, liver, heart, and lung. Numerous clinical studies have demonstrated that I-postC can induce endogenous protective mechanisms, reduce endothelial dysfunction, decrease apoptosis rates, and reduce ischemic necrotic areas, thereby alleviating tissue ischemia-reperfusion injury. The mechanisms underlying these effects have become a research hotspot. Although studies on the protective effects of ischemic postconditioning on organs such as the heart and kidney have gradually increased, the relationship between I-postC and pressure ulcer ischemia-reperfusion injury remains unclear, and the effective time window for I-postC' s protective effect on pressure ulcer ischemia-reperfusion injury has not been reported. Based on an established rat model of pressure ulcer ischemia-reperfusion injury, this study investigated the effects of ischemic postconditioning on pressure ulcers induced at different time windows to identify the optimal time window for its protective effect.

Materials and Methods

1.1.2 Main Instruments

Ultra-clean workbench (Animal Laboratory of Sun Yat-sen University North

Campus), electronic balance, air compressor (USA), camera, centrifuge (Germany), HARRIS INC ultra-low temperature freezer (USA), BX50-OLYMPUS optical microscope (Japan), and a set of surgical instruments (Shanghai Jinli Medical Instruments).

1.1.3 Main Reagents

10% chloral hydrate, 4% paraformaldehyde fixative, 4% glutaraldehyde fixative, phosphotungstic acid hematoxylin staining kit, fluorescent immunohistochemistry monitoring kit (SP-9002) (Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.); neutrophil infiltration detection kit (Biyuntian Biotechnology).

1.2.1 Animal Model Preparation

Healthy adult male SD rats were selected to create an ischemia-reperfusion pressure ulcer model. Rats were anesthetized by intraperitoneal injection of 10% chloral hydrate at a dose of 400 mg/kg. An area of approximately 2 cm × 2 cm on the thigh gracilis muscle was selected and leg hair was clipped. Rats were placed prone on the compression device with limbs fixed by adhesive tape. The air compressor was connected to the pressure cylinder, and the pressure contact surface was applied vertically to the rat' s thigh gracilis muscle at 412.5 mmHg for 2 hours. After successful modeling and completion of compression and postconditioning, the compressed central skin and muscle tissue of the gracilis muscle (approximately 0.5 cm × 0.5 cm × 0.5 cm) was excised under anesthesia for 制片观察. Abdominal aortic blood (2 mL) was randomly collected, placed in Ep tubes, centrifuged at 3600 r/min for 10 minutes at 4°C, and plasma was collected and stored at -80°C for later use. Rats were finally euthanized by air embolism.

1.2.2 Ischemic Postconditioning

After reaching the compression ischemia time of 2, 4, 6, or 8 hours, pressure was released and immediate intermittent ischemia was applied with 10 minutes decompression/10 minutes compression for three cycles, with one processing time totaling 1 hour.

1.2.3 Animal Grouping

Sixty-eight SD rats were randomly divided into 9 groups using a random number table: a blank control group (Group S, n=4) receiving no intervention, and experimental groups with 8 rats each. Group a: 2-hour compression ischemia; Group b: 2-hour compression ischemia + immediate ischemic postconditioning; Group a: 4-hour compression ischemia; Group b: 4-hour compression ischemia + immediate ischemic postconditioning; Group a: 6-hour compression ischemia; Group b: 6-hour compression ischemia + immediate ischemic postconditioning (anesthetized and fixed prone on the rat board with continuous compression for 6 hours followed by immediate postconditioning after decompression); Group a: 8-hour compression ischemia; Group b: 8-hour compression ischemia + immediate ischemic postconditioning.

1.2.4 Observation Indicators

1.2.4.1 Measurement of Skin and Subcutaneous Tissue Damage Severity

Tissue samples were stained with PTAH (phosphotungstic acid hematoxylin). Specific staining steps: tissue blocks were obtained, fixed, routinely paraffin-embedded, and sectioned at 4 m. Referring to the method of Wang Yan et al., rat skin damage was classified into three grades: mild, moderate, and severe. Mild damage: thinning of squamous epithelial cells, loose connection between epidermis and dermis, mild collagen degeneration in dermis, capillary dilation, and minimal lymphocyte infiltration. Moderate damage: partial nuclear pyknosis in squamous epithelium, partial separation of epidermis and dermis, collagen fiber degeneration in dermis, obvious inflammatory cell infiltration, free fat cells, capillary dilation and congestion, striated muscle edema, and widened interstitial spaces. Severe damage: squamous epithelial desquamation, separation of epidermis and dermis, dissolution of dermal collagen fibers, nuclear fragmentation, dissolution of fat cells, capillary congestion, striated muscle degeneration, and blurred striations.

1.2.4.2 Measurement of Neutrophil Infiltration

Using the HE method, neutrophil infiltration in skin was graded into 4 levels. The staining procedure was as follows: (1) Tissue blocks were obtained, fixed, routinely paraffin-embedded, and sectioned at 4 m. (2) Sections were routinely deparaffinized with xylene. (3) Hematoxylin staining for 5 minutes, rinsed with tap water. (4) Differentiation with hydrochloric acid ethanol for 30 seconds (dipped several times). (5) Soaked in tap water for 15 minutes or warm water (approximately 50°C) for 5 minutes. (6) Placed in eosin solution for 2 minutes. (7) Routine dehydration, clearing, and mounting.

1.2.4.3 Determination of Serum Superoxide Dismutase (SOD) Activity

SOD can scavenge superoxide anion free radicals, protect cells from damage, and plays a crucial role in maintaining the oxidative and antioxidant balance in the body. SOD activity was measured using the xanthine oxidase method. Absorbance was measured at 550 nm using a 722 spectrophotometer, and SOD activity was calculated using a formula. Enzyme activity was defined as the amount of SOD corresponding to 50% inhibition rate per milliliter of reaction solution.

1.2.4.4 Determination of Serum Malondialdehyde (MDA) Content

MDA is a product of lipid peroxidation reactions, and its content in serum and tissues indirectly reflects the degree of free radical damage to tissues. Its quantity can reflect the speed and intensity of lipid peroxidation reactions in tissues. Absorbance was measured at 532 nm using a 722 visible spectrophotometer, and content was calculated using a formula.

1.2.4.5 Determination of Serum Nitric Oxide (NO) Content (Nitrate Reductase Method)

NO is a highly reactive free radical in the body with extensive physiological effects, such as relaxing vascular smooth muscle, inhibiting platelet aggregation, and preventing atherosclerosis. Tissue injuries like pressure ulcers can affect NO generation in blood circulation. NO content was measured using the nitrate reductase colorimetric method. NO reacts with oxygen and water to generate nitrate and nitrite, which react with nitrate chromogenic agents to produce a light red azo compound. Absorbance was measured at 550 nm using a 722 visible spectrophotometer to detect serum NO content.

1.3 Statistical Methods

The rank-sum test for ordered polytomous data was used to compare pressure ulcer incidence and skin compression severity. Intergroup comparisons were performed using one-way ANOVA, with Dunnett' s t-test for pairwise comparisons between experimental and control groups. Data were expressed as mean ± standard deviation, with $P<0.05$ considered statistically significant. All data were processed using SPSS 16.0 statistical software.

Results

2.1 Relationship Between Different Intervention Modalities and Injury Severity

In Group b (compression + postconditioning), 17 rats showed mild skin injury, 10 showed moderate injury, and 4 showed severe injury. In Group a (compression only), the corresponding numbers were 14, 14, and 4. The rank means for injury severity were 3, 38, and 34 for the three groups, with the difference in distribution being statistically significant ($P=0.001$, Table 1).

Table 1 Comparison of skin lesion severity among different groups (n)
Damage degree: Normal | Moderate | Severe
Group IR (a): [data]
Group I-PostC (b): [data]
Total Rank range: 37~60 | 61~68

2.2 Relationship Between Compression Time and Injury Severity

Two rats in the 6-hour compression group exhibited severe damage, while 6 rats (37.5%) in the 8-hour group showed severe damage. No severe damage occurred in the 2-hour or 4-hour compression groups. Regarding injury severity, 4-hour and 6-hour compression primarily caused mild and moderate skin injury [both n=15 (9+6 and 7+8 respectively)]. Statistical analysis revealed rank means of 25, 29, 36, and 40 for different compression times, with the difference in distribution being statistically significant ($P=0.043$, Table 2).

Table 2 Comparison of skin lesion severity among different compression time windows
Damage degree: Normal | Moderate | Severe
Compression time: [data]
Total Rank range: 33~56 | 57~64

2.4 Effect of Compression Time on Neutrophil Infiltration Degree

Groups a and b predominantly showed Grade I infiltration (Figure 1 [Figure 1: see original paper] and Figure 2). Group b had more rats with Grade I infiltration (mildest degree) than Group a (n=17 vs. n=15), and fewer with Grade II infiltration (n=8 vs. n=10). Grade III infiltration was also less frequent in Group b than in Group a (n=1 vs. n=2). Statistical analysis revealed rank means of 3, 37, and 36 for neutrophil infiltration degree among the three groups, with significant differences (P=0.002, Table 3).

Among the 64 model rats, Grade I infiltration predominated (n=32 and n=18), followed by Grade II (n=11 and n=3). Two-hour compression caused the mildest infiltration, followed by 4-hour and 6-hour compression, while 8-hour compression caused the most severe infiltration, with 6 rats (37.5%) reaching Grade IV. Statistical analysis revealed rank means of 24, 29, 35, and 42 for neutrophil infiltration degree across different compression times, with significant differences (P=0.027, Table 4).

Figure 1 Grade of neutrophil infiltration. A: Grade I neutrophil infiltration (HE staining, original magnification: $×$20); B: Grade II neutrophil infiltration (DAB staining and hematoxylin counterstaining, $×$20).

Table 3 Comparison of neutrophil infiltration in compressed skin and subcutaneous tissue among different groups
Neutrophil infiltration grade: Normal | Grade I | Grade II | Grade III | Grade IV
Group IR (a): [data]
Group I-PostC (b): [data]
Total Rank range: 37~54 | 55~65 | 66~68

Table 4 Comparison of neutrophil infiltration in compressed skin and subcutaneous tissue among groups with different compression time
Neutrophil infiltration grade: Normal | Grade I | Grade II | Grade III | Grade IV
Compression time: [data]
Total Rank range: 33~50 | 51~61 | 62~64

2.6 Differences in MDA Content Under Different Intervention Modalities

With prolonged intervention time, both Groups a and b showed decreased SOD content, with statistically significant gradient differences (P=0.02 and P=0.007 respectively). SOD levels at 2, 4, and 6 hours were significantly higher than at 8 hours; 2-hour and 4-hour values were higher than 6-hour values, while no significant difference existed between 2-hour and 4-hour values. Intergroup comparison revealed that SOD content in Group b was significantly higher than in Group a at 2, 4, and 6 hours (P<0.05), but no significant difference was observed at 8 hours (Table 5).

With prolonged intervention time, both Groups a and b showed increased MDA content, with statistically significant gradient differences (P<0.001). MDA lev-

els at 2, 4, and 6 hours were significantly lower than at 8 hours; 2-hour values were significantly lower than 4-hour and 6-hour values, while no significant difference existed between 4-hour and 6-hour values. Intergroup comparison revealed that MDA content in Group b was significantly lower than in Group a at 2, 4, and 6 hours (P<0.05), but no significant difference was observed at 8 hours (Table 6).

Table 5 Comparison of serum SOD activity in rats with different compression time (U/mL, Mean±SD)
Compression time (h): 2 | 4 | 6 | 8
Group IR (a) (n=4$×8 = 32) : 118.48±3.67*#|113.68±5.51*#|102.68±4.98▲|89.68±4.98▲*
$GroupI - PostC(b)(n = 4×8 = 32)$: 123.45±5.12 * #|122.68±5.52 * #|110.68±5.53▲|90.08±$5.98 *
Compare to group 4 h, P<0.05; Compare to group 6 h, *P<0.05; Compare to group 8 h, #P<0.05.

Table 6 Comparison of serum MDA levels in rats with different compression time (nmol/mL, Mean±SD)
Compression time (h): 2 | 4 | 6 | 8
Group IR (a) (n=4$×8 = 32) : 5.67±0.46▲*#|7.33±0.53#|7.23±0.41#|9.99±0.67▲*
$GroupI-PostC(b)(n = 4×8 = 32)$: 4.88±0.66*#|5.23±0.41#|6.13±0.67#|10.23±$0.19 *
Compare to group 4 h, P<0.05; Compare to group 6 h, *P<0.05; Compare to group 8 h, #P<0.05.

2.7 Differences in NO Content Under Different Intervention Modalities

With prolonged intervention time, both Groups a and b showed increased NO content, with statistically significant gradient differences (P<0.001). NO levels at 2, 4, and 6 hours were significantly lower than at 8 hours; 2-hour and 4-hour values were significantly lower than 6-hour values, while no significant difference existed between 2-hour and 4-hour values. Intergroup comparison revealed that NO content in Group b was significantly lower than in Group a at 2, 4, and 6 hours (P<0.05), but no significant difference was observed at 8 hours (Table 7).

Table 7 Comparison of serum NO levels in rats with different compression time (mol/L, Mean±SD)
Compression time (h): 2 | 4 | 6 | 8
Group IR (a) (n=4$×8 = 32) : 41.58±1.12 * #|48.12±1.52▲|56.12±1.32▲ * |66.12±1.32▲ * $GroupI - PostC(b)(n = 4×8 = 32)$: 39.35±1.26 * #|45.12±1.87▲|55.32±1.85▲ * |65.32±$1.85 *
Compare to group 4 h, P<0.05; Compare to group 6 h, *P<0.05; Compare to group 8 h, #P<0.05.

Discussion

Ischemia-reperfusion injury (IRI) is a phenomenon where tissue and organ damage is further exacerbated by the restoration of blood flow after a period of ischemia. Recent studies have indicated that IRI has become a major determinant

of pressure ulcer formation. During the ischemia-reperfusion process of pressure ulcers, altered blood flow patterns and vortex phenomena lead to microcirculatory disturbances including vascular endothelial dysfunction, vasoconstriction, increased blood viscosity, enhanced red blood cell aggregation, slowed blood flow, white microthrombus formation, and the no-reflow phenomenon. Donato-Trancoso et al. found that free radicals generated during ischemia-reperfusion are associated with cellular damage and pressure ulcer development. Other studies have demonstrated that the protective effect of ischemic postconditioning is comparable to that of ischemic preconditioning, but with better controllability since it does not require prediction of ischemic events, thus offering broader application scope and practical value. By inhibiting the burst production of free radicals after ischemia-reperfusion, ischemic postconditioning prevents direct free radical damage to tissues and reduces neutrophil participation, thereby exerting protective effects on various tissues susceptible to ischemia-reperfusion injury. Ischemic postconditioning has been successfully applied in experimental and clinical studies of reperfusion injury in brain, intestine, spinal cord, kidney, liver, heart, and lung. International studies using a rat middle cerebral artery occlusion model have shown that ischemic postconditioning before reperfusion significantly reduced cerebral infarct area. Studies on the protective effects of hepatic ischemic postconditioning have also confirmed its protective role against liver ischemia-reperfusion injury. Similar results have been obtained in studies investigating the protective effect of ischemic postconditioning on intestinal mucosal injury in ischemia-reperfusion rats. Research has confirmed that ischemic postconditioning can improve functional recovery after injury in heart, brain, kidney, and skeletal muscle, reduce oxygen free radicals and lipid reactions, decrease excessive inflammatory responses, and mitigate tissue cell damage caused by apoptosis and vascular endothelial cell dysregulation, though treatment protocols and efficacy require further exploration.

Our previous research found that ischemic postconditioning significantly alleviated 2-hour injury in a rat skeletal muscle ischemia-reperfusion pressure ulcer model. Both IR and I-postC groups showed 100% pressure ulcer incidence at the same compression time. The I-postC group showed serum NO and MDA levels higher than Group S but significantly lower than the IR group, while SOD activity was lower than Group S but significantly higher than the IR group. These findings indicated that skeletal muscle damage caused by ischemia began to recover gradually after 2 hours, with faster recovery in the I-postC group than in Group S, demonstrating that ischemic postconditioning provides long-term protection against focal ischemia and that functional recovery does not necessarily depend solely on shortening compression time. Building on our previous research, this study added observations of ischemic postconditioning effects on pressure ulcer injury across different time windows. We found that under identical methods and compression times, significant differences existed between IR and I-postC groups in skin and subcutaneous tissue damage, neutrophil infiltration degree, and serum SOD activity, MDA, and NO content at 2, 4, and 6 hours of compression. These results demonstrate that ischemic postconditioning

exerts protective effects in ischemia-reperfusion pressure ulcers within a certain time frame.

Clinically, patients who are bedridden or sit for prolonged periods are highly susceptible to pressure ulcers. No clear guidelines exist for patient turning intervals, though typically patients are turned every 2 hours. ICU patients at high risk for pressure ulcers using air mattresses are turned every 4 hours, and elderly patients using a gradual extension of compression intervals (30-minute increments per stage) show lower pressure ulcer incidence than conventional 2-hour turning groups. While these methods provide some preventive effect for certain patients, systematic studies on long-term bedridden patients are lacking, and baseline consistency among study subjects and the effective time window for ischemic treatment remain undefined. Based on these studies, we selected ischemic treatment times of 2, 4, 6, and 8 hours, consistent with our previous research findings. This study found that even under the same time and ischemia-reperfusion cycle conditions, rats exhibited different injury outcomes, indicating individual differences in skin tolerance among rats. Under identical methods and compression times, significant differences existed between IR and I-postC groups in skin and subcutaneous tissue damage, neutrophil infiltration degree, and SOD activity, MDA, and NO content at 2, 4, and 6 hours of compression. However, no significant differences were observed between IR and I-postC groups at 8 hours of compression, indicating that while ischemic postconditioning provides protective effects in ischemia-reperfusion pressure ulcers within a certain time window, this protection is not effective at any compression duration. Continuous compression exceeding 8 hours causes irreversible damage within a short period, rendering subsequent ischemic postconditioning clinically meaningless. The same principle applies to clinical patients—bedridden patients with thin subcutaneous fat and malnutrition require frequent observation and turning, with turning schedules tailored to individual physical conditions. Among the four ischemic time points selected in this study (2, 4, 6, and 8 hours), analysis of all experimental results indicates that ischemic postconditioning provides protective effects for rats undergoing 2, 4, and 6 hours of ischemia with reperfusion. However, the protective effect of ischemic postconditioning on rat ischemia-reperfusion injury gradually diminishes and eventually disappears with prolonged ischemia duration.

Based on these experimental results, we conclude that: (1) Ischemic postconditioning can alleviate ischemia-reperfusion pressure ulcers in rats, as evidenced by reduced skin color changes, diminished inflammatory cell aggregation, decreased oxygen free radical alterations, and improved functional deficits, but this protective effect is limited by skeletal muscle ischemia duration and gradually weakens with prolonged ischemia; (2) The effective time window for ischemic postconditioning' s protective effect on rat skeletal muscle ischemia-reperfusion injury is within 6 hours of ischemia, with optimal protection within 4 hours.

This study originated from basic research, establishing a pressure ulcer model in rats using a pressure device based on referenced animal model preparation

methods. We investigated the causes of pressure ulcer formation, histopathological changes, and biochemical indicator variations during pressure ulcer development, exploring skin injury mechanisms at the cellular ultrastructure and molecular levels. Building on this foundation, we examined the protective effects of ischemia-reperfusion postconditioning on pressure ulcers induced at different time windows to determine the optimal protective timing. These findings provide important references for pressure ulcer management and hold clinical significance for nursing practice.

References

[1] Oliveira CC, Sabino PP, Ad AS, et al. Pressure ulcer: incidence and demographic, clinical and nutrition factors associated in intensive care unit patients[J]. Nutricion Hospitalaria, 2015, 32(5): 2242.

[2] Posthauer ME, Banks M, Dorner B, et al. The role of nutrition for pressure ulcer management: National pressure ulcer advisory panel, European pressure ulcer advisory panel, and Pan pacific pressure injury alliance White Paper[J]. Adv Skin Wound Care, 2015, 28(4): 175-88; quiz 189-90.

[3] Ferdinandy P, Hausenloy DJ, Heusch G, et al. Interaction of risk factors, comorbidities, comedications with ischemia/ reperfusion injury and cardioprotection by preconditioning, postconditioning, and remote conditioning[J]. Pharmacol Rev, 2014, 66(4): 1142-74.

[4] Liao XX, Wang LC, Yang CT, et al. Cyclooxygenase mediates cardioprotection of angiotensin- (1-7) against ischemia/reperfusion-induced injury through the inhibition of oxidative stress[J]. Mol Med Rep, 2011, 4(6): 1145-50.

[5] Buchholz B, Donato M, D' annunzio V, Ischemic postconditioning: mechanisms, comorbidities, clinical application[J]. Mol Cell Biochem, 2014, 392(1/2): 1-12.

[6] Csonka C, Szucs G, Varga-Orvos Z, et al. Ischemic postconditioning alters the gene expression pattern of the ischemic heart[J]. Exp Biol Med, 2014, 239(2): 141-50.

[7] Lishmanov YB, Gorbunov AS, Maslov LN. Involvement of protein kinase c-delta in the realization of cardioprotective effect of ischemic postconditioning[J]. Bull Exp Biol Med, 2016, 161(3): [page numbers].

[8] Cui FF, Pan YY, Xie HH, et al. Pressure combined with ischemia/reperfusion injury induces deep tissue injury via endoplasmic reticulum stress in a rat pressure ulcer model[J]. Int J Mol Sci, 2016, 17(3): 453.

[9] Huang F, Yang JJ, Wang XL, et al. Expression and significance of VEGF and bFGF in a rat pressure ulcer model[J]. Chinese Journal of Pathophysiology, 2012, 28(8): 1504-6.

[10] Zhang ZZ, Luo X, Yu XH. Astragalus injection inhibits inflammatory response and enhances antioxidant capacity in rats with pressure ulcer ischemia-

reperfusion injury[J]. Chinese Journal of Cellular and Molecular Immunology, 2015, 31(4): 507-10.

[11] Wang XH, Mao TT, Pan YY, et al. Expression and significance of TNF-α, MMP-2 and collagen in pressure ulcer skin tissue of rats[J]. Chinese Journal of Burns, 2016, 32(3): 160-7.

[12] Wang Y, Diao B, Zheng GR, et al. Effects of ω-3 polyunsaturated fatty acids on tissue injury in aged rats[J]. Herald of Medicine, 2013, 32(2): 139-42.

[13] Deng YH, Qin HY, Wen G, et al. Effects of Huayu Jiedu method on thrombin and its receptor, MCP-1 and NF- B expression, and neutrophil infiltration in MCAO rats[J]. World Chinese Medicine, 2016, 11(4): 575-9.

[14] Kasuya A, Sakabe JI, Tokura Y. Potential application of in vivo imaging of impaired lymphatic duct to evaluate the severity of pressure ulcer in mouse model[J]. Sci Rep, 2014, 4(8): 4173.

[15] Xiao HL, Long ZJ, Shi H, et al. Effects of pressure ulcer remedy on local expression of SOD, MDA, VEGF and EGF in a rat pressure ulcer model[J]. Pharmacology and Clinics of Chinese Materia Medica, 2016(1): 137-40.

[16] Avci E, Akarslan ZZ, Erten H, et al. Oxidative stress and cellular immunity in patients with recurrent aphthous ulcers[J]. Braz J Med Biol Res, 2014, 47(5): 355-60.

[17] Lan YS, Gao W, Liao HT, et al. Expression and role of superoxide dismutase, malondialdehyde and endothelin in a rat pressure ulcer model[J]. Journal of Nursing, 2017, 24(9): 1-6.

[18] Azadi S, Ashrafi H, Azadi A. A hopeful prospect of riociguat as a soluble guanylate cyclase stimulator for management of pressure ulcers[J]. Curr Drug Discov Technol, 2017, 60(12): 78.

[19] Lu YW, Ji B, Dai J, et al. Effects of different frequencies of electroacupuncture and stimulation times on skin blood perfusion at Neiguan point and non-meridian non-acupoint points in myocardial ischemia rats[J]. Journal of Beijing University of Traditional Chinese Medicine, 2016, 39(12): 1041-7.

[20] Wang YY, Pu LH, Li Z, et al. Hypoxia-Inducible factor-1 alpha gene expression and apoptosis in Ischemia-Reperfusion injury a rat model of Early-Stage pressure ulcer[J]. Nurs Res, 2016, 65(1): 35-42.

[21] Gust MJ, Hong SJ, Fang RC, et al. Adipose tissue drives response to Ischemia-Reperfusion injury in a murine pressure Sore model[J]. Plast Reconstr Surg, 2017, 139(5): 1128E-38E.

[22] Donato-Trancoso A, Monte-Alto-Costa A, Romana-Souza B. Olive oil-induced reduction of oxidative damage and inflammation promotes wound healing of pressure ulcers in mice[J]. J Dermatol Sci, 2016, 83(1): 60-9.

[23] Talab SS, Emami H, Elmi A, et al. Chronic Lithium treatment protects the rat kidney against ischemia/reperfusion injury: The role of nitric oxide and cyclooxygenase pathways[J]. Eur J Pharmacol, 2010, 647(1/3): 171-7.

[24] Andreas M, Schmid AI, Keilani MA, et al. Effect of ischemic preconditioning in skeletal muscle measured by functional magnetic resonance imaging and spectroscopy: a randomized crossover trial[J]. J Cardiovasc Magn Reson, 2011, 13(1): 1-10.

[25] Santos CH, Aydos RD, Nogueira NE, et al. Importance of duration and number of ischemic postconditioning cycles in preventing reperfusion mesenteric injuries. Experimental study in rats[J]. Acta Cirurgica Brasileira, 2015, 30(10): 709-14.

[26] Zhao H, Sapolsky RM, Steinberg GK. Interrupting reperfusion as a stroke therapy: ischemic postconditioning reduces infarct size after focal ischemia in rats[J]. J Cereb Blood Flow Metab, 2006, 26(9): 1234-41.

[27] Wassermann E, van Griensven M, Gstaltner KA, et al. A chronic pressure ulcer model in the nude mouse[J]. Wound Repair Regen, 2009, 17(4): 480-4.

[28] Lin F, Pandya A, Cichowski A, et al. Deep tissue injury rat model for pressure ulcer research on spinal cord injury[J]. J Tissue Viability, 2010, 19(2, SI): 67-76.

[29] Wang YY, Sun HQ, Zhang CY, et al. Preparation and evaluation of an early pressure ulcer animal model[J]. Journal of Nursing, 2010, 25(14): 1-5.

[30] Siu PM, Tam EW, Teng BT, et al. Muscle apoptosis is induced in pressure-induced deep tissue injury[J]. J Appl Physiol, 2009, 107(4): 1266-75.

[31] Rentoukas I, Giannopoulos G, Kaoukis AA, et al. Cardioprotective role of remote ischemic periconditioning in primary percutaneous coronary intervention enhancement by opioid action[J]. JACC Cardiovasc Interv, 2010, 3(1): 49-55.

Note: Figure translations are in progress. See original paper for figures.