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Effect of Human Placental Extract Treatment on Random-Pattern Skin Flap Survival in Rats

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ABSTRACT

Background: Human placental extract (HPE), prepared from the placentas of healthy, postpartum females, displays various physiological activities, including antioxidative properties. In this study, a dorsal skin flap model was used to investigate the effect of HPE on flap viability in rats. *Materials and methods*: Forty male Sprague-Dawley rats underwent random-pattern skin flap surgeries. The animals were randomly divided among a control group and three treatment groups (localized injection (LI), 10 mg/kg/d localized HPE injections; low-dose treatment (LT), 10 mg/kg/d systemic HPE injections; high-dose treatment (HT), 40 mg/kg/d systemic HPE injections). Surviving skin flap areas were measured 7 days after surgery and tissue samples were stained with hematoxylin and eosin; vascular endothelial growth factor expression was determined immunohistochemically. To evaluate the antioxidant and antiapoptotic effects of HPE, malondialdehyde, glutathione peroxidase, and caspase-3 levels were examined. *Results*: Seven days after surgery, HPE-treated animals had significantly reduced necrotic areas, rats receiving the highest HPE dose demonstrated the greatest flap survival. In the HPE groups, the histopathological scores were lower than for the control group. Immunohistochemistry showed markedly more numerous vascular endothelial growth factor-positive cells in the HT group than in the C group. Malondialdehyde levels were significantly lower and glutathione peroxidase levels were higher in the HT group than in the C group. HPE treatment significantly inhibited apoptosis by lowering caspase-3 activity. *Conclusions*: HPE treatment yielded positive effects on flap survival, due to its antioxidant and antiapoptotic properties. These results suggest a new therapeutic approach for enhancing flap viability and accelerating wound repair.

Keywords: human placental extract; random-pattern flap; antioxidant

INTRODUCTION

Random-pattern skin flaps remain a widely used reconstructive option for covering both acquired and congenital defects. However, distal flap loss is a common complication resulting from inadequate blood supply, inflammatory reactions, and oxidative stress. This type of ischemic injury leads to clogging of the microvasculature with neutrophils and the potential release of free radicals, enzymes, and cytokines. These, in turn, physically injure the endothelium, and obstruct the capillaries, blocking oxygen supply to the tissue.[1, 2] Thus, the basic components contributing to the tissue damage are oxygen radicals and proinflammatory cytokines/chemokines.[3] To increase flap survival, a number of strategies have been described to enhance the blood supply and prevent free radical formation.[4, 5] Despite the numerous advances in endovascular procedures, surgical bypass approaches, and wound-healing research, ischemic nonhealing wounds continue to be clinically challenging; impaired tissue oxygen levels and neovascularization remain serious unsolved problems.[6] Therefore, various therapeutic approaches continue to be pursued to prevent ischemia-induced tissue damage through the use of nonspecific local or systemic physical stressors and pharmacological

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agents.[7,8] Previous research has shown that systemic and/or local drug agents, such as alpha-adrenergic blockers, phenothiazines, vasodilators, and various angiogenic growth factors, can help overcome flap loss.[9,10]

The biological actions induced by human placental extract (HPE) constitute a topic of increasing interest.[11] Reportedly, HPE constituents are diverse and include large numbers of biologically active molecules, including RNA, DNA, peptides, amino acids, proteins, lipids, and enzymes.[12–14] Moreover, a number of growth factors and their receptors have also been identified, including hepatocyte growth factor [15], epidermal growth factor [16], transforming growth factor-b [17], and transforming growth factor-α.[18] Many of these agents are believed to act in an autocrine/paracrine fashion within the human placenta, regulating the production of other biologically active substances through a complex interplay of potentially therapeutic factors. Because of its strong antioxidant substances, antiinflammatory mediators, and growth factors, HPE may play a potential role in protecting liver cells from a variety of injuries.[19–22] Laennec (Green Cross, Japan Bio Products, Gyeonggi, Korea) is an HPE prepared using dialysis, heat treatment, and hydrolysis.[23] The role of this HPE in flap survival has not been assessed; we hypothesized that HPE might act as both a strong free radical scavenger and an angiogenesis enhancer, reducing flap ischemic injury during surgery. Therefore, we investigated the protective effects of this commercially available HPE on flap survival in a classic rat model of random-pattern dorsal flaps. Our experience and results are discussed

MATERIAL AND METHODS

Animals

A total of 40 male Sprague-Dawley rats, weighing 200–250 g, were randomly divided into four equally sized groups. The animals were housed at a constant room temperature (21 ± 220 C), and were individually housed, after surgery, to prevent cannibalization. Animals were euthanized, on postoperative day 7, using carbon dioxide. The animal protocols were approved by the Ewha Womans University Animal Care Committee.

Flap Model

Animals were anesthetized using an intraperitoneal dose of tiletamine/zolazepam (Zoletil 50, Virbac, Carros, France; 0.1 mL/100 g body weight). A preoperative, intramuscular dose of cefazolin sodium (0.1 mg/kg) was also administered for infection prophylaxis. Prior to surgery, the dorsal skin of each animal was shaved and cleansed using povidone-iodine. The same surgeon created a cranially based skin flap (10 × 3 cm) on the shaved dorsum of each rat. The flap was elevated beneath the panniculus carnosus using the McFarlane's skin flap model.[24] The palpable hip joints were used as anatomical landmarks to define the flap base. When perforating vessels were found in the flap base, the vessels were electrically cauterized to create flaps with a completely random vascular pattern. Each flap was then sutured back to its donor site using 4-0 black silk. Following surgery, the rats in the treatment groups received daily (days 0–6) HPE administrations.

The four experimental groups included a control group (C) that received daily intraperitoneal injections of the vehicle (0.9% saline, 0.1 mL). The localized injection (LI) group received daily subcutaneous HPE injections (0.1 mL/d), localized to the proximal area of the flap. The low-dose treatment (LT) group received daily intraperitoneal injections of the HPE solution (0.1 mL/d), and the high-dose treatment (HT) group received similar injections at a higher dosage (0.3 mL/d). A single subcutaneous dose of carprofen (5 mg/kg) was administered, immediately after surgery, as an analgesic.

Measurement of the Flap Survival Rate

All of experimental animals survived throughout the experimental period. On postsurgical day 7, the necrotic skin flap areas were photographed (Canon 70D, Canon, Tokyo, Japan) from a distance of 20 cm while the rats were anesthetized. The necrosis/total flap ratio of each flap was analyzed using image analysis software (ImageJ, National Institutes of Health, Bethesda, MD, USA). Dark and stiff zones of the flaps were defined as being necrotic; the remaining areas were defined as surviving areas. The total and necrotic areas of each flap were measured, and skin flap survival was expressed as a percentage of the total skin flap area (Figure 1).

Histologic Assessment

On postsurgical day 7, flap specimens (1 × 1 cm) were taken from the centers of the surviving flap areas of all animals, 1 cm from the necrotic area. All specimens were fixed in 10% buffered formalin for at least 24 h, and then embedded in paraffin. Each tissue specimen was sectioned (5 μm thick) and stained with hematoxylin and eosin for routine histopathological observation. Specimens were assessed using a semiquantitative histopathologic scoring system based on degree of inflammation (polymorphonuclear leukocyte and lymphocyte density), dermal edema

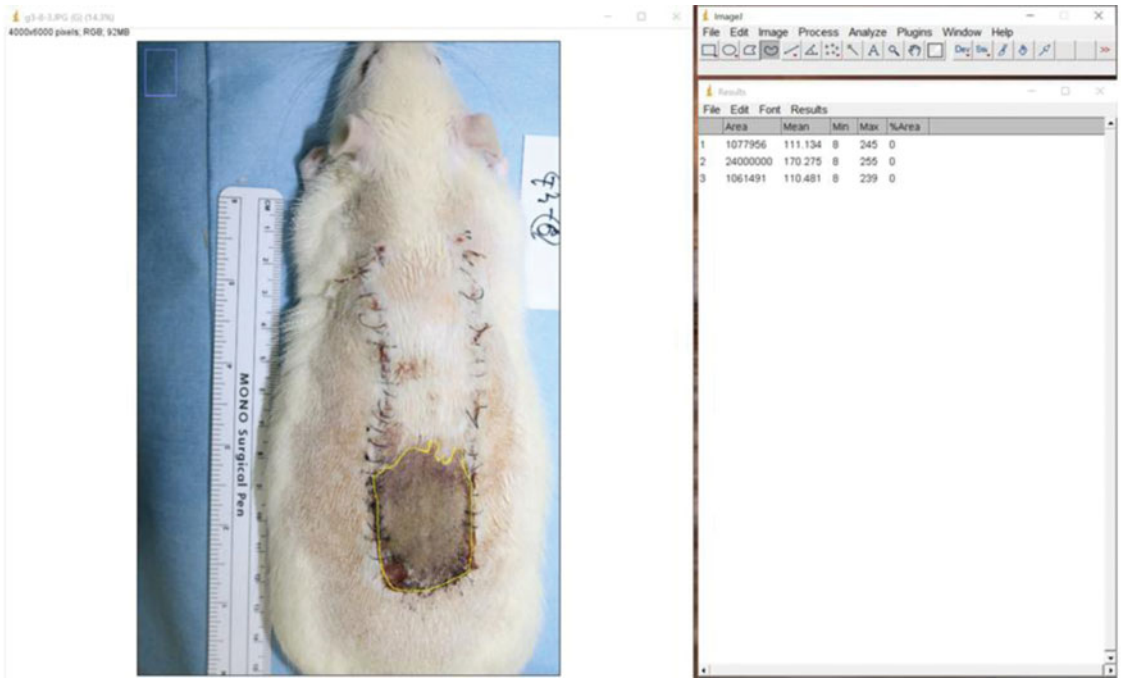

FIGURE 1 Flap surface area measurement using image analysis software.

(separation from the reticular dermis), and collagen density (Table 1). The scoring was performed by two histopathologists, blinded to the treatment groups, using high-power (200×) magnification. The pathological score for each tissue was calculated by averaging the scores of the three parameter scores. According to this scoring system, higher scores indicated more severe tissue damage.

Immunohistochemical Evaluation

Vascular endothelial growth factor (VEGF) expression levels in the tissues of animals from the four groups were assessed immunohistochemically. Tissue samples (paraffin-embedded, 4-µm thick) were stained using a primary antibody against rat VEGF (Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:100 dilution for 1 h at room temperature) and a secondary antirat antibody (UltraVision LP Detection System, Thermo Scientific, Pittsburgh, MA, USA; 35 min at room temperature). The 3-amino-9-ethyl carbazole substrate system (Spring Bioscience, Lab Vision, Fremont, CA, USA) was used for color development. Lastly, the sections were counterstained, using Mayer's hematoxylin, and mounted. Regions of intense VEGF staining were located under low magnification, and the number of VEGF-positive microvessels (VEFG score) were quantified in at least five fields, per specimen, under high magnification (200×). The two pathologists conducting the assessment were blinded to specimen identities.

TABLE 1 Histopathologic scoring system.

Score	0	1	2	3
Inflammation	None	Some	Moderate	Severe
Dermal edema	Normal	Mild	Moderate	Extensive
Collagen density	Normal	Mild	Moderated	Extensive

Biochemical Analysis

To evaluate ischemia and free radical damage, biopsies (1 × 1 cm) were taken, starting 3 cm distal to the flap base, from animals in each group. Each specimen was weighed using a microbalance, and trimmed to a uniform weight for analysis.

Glutathione Peroxidase (GPx) Determination

GPx is one of the enzymes for endogenous antioxidant defense systems. In order to obtain GPx, skin samples (20 mg) were homogenized, on ice, in 1.5-mL tubes containing GPx assay buffer. After cooling, the samples were centrifuged (10,000 × g, 15 min, 4°C) using a microcentrifuge (5424 R, Eppendorf, Hamburg, Germany). The supernatants were collected, and GPx activities were estimated by measuring the optical densities (340 nm) of the samples in a multimode microplate reader (BioTek Instruments, Winooski, VT, USA), according to the kit directions (Glutathione

Peroxidase Activity Assay Kit, K762-100, BioVision Research Products, Mountain View, CA, USA).

Malondialdehyde Determination

Malondialdehyde (MDA) represents the end product of lipid peroxidation. To determine MDA levels, tissue (10 mg) was homogenized, on ice, in MDA Lysis Buffer (300 μL) and butylhydroxytoluene (100×, 3 μL), and centrifuged (13,000 × g, 10 min, 4°C) to remove insoluble material. MDA levels were then determined using the thiobarbituric acid method (MDA Assay Kit, K739-100, BioVision Research Products). Briefly, MDA quantification involved a fluorometric analysis (excitation wavelength, 532 nm; emission wavelength, 553 nm) in a multimode microplate reader (BioTek Instruments), according to the manufacturer's instructions.

Caspase-3 Determination

Caspase-3 is an IL-converting enzyme that has been suggested to be the principal effector of mammalian apoptotic and inflammatory pathways.[22] An essential component of the apoptotic transduction pathway, triggered by ischemia, is the intracellular generation of reactive oxygen species and regulated by bax/blc-2 gene expressions. The assay involves the use of a luminescent substrate containing the caspase substrate peptide, Asp(Aspartic acid)-Glu(Glutamic acid)-Val (Valine)-Asp(Aspartic acid) (DEVD), in a reagent optimized for caspase and luciferase activities. Following cleavage of the substrate by caspase-3 and caspase-7, aminoluciferin is released, resulting in luciferase-based light production. Tissue (10 mg) was homogenized, on ice, in caspase-3 lysis buffer and centrifuged (10,000 × g , 1 min, 4°C) in a microcentrifuge. Supernatant protein levels were determined using the bicinchoninic acid method, and the samples diluted to a final protein concentration of 1 μg/μL. The diluted samples were incubated with the caspase substrate (10 μL) for 3 h at room temperature. Finally, sample luminescence was measured in a multimode microplate reader (BioTek Instruments).

Statistical Analysis

Statistical analyses were performed using SPSS software, version 22.0 (IBM, Armonk, NY, USA). Differences between the groups were analyzed using the Kruskal–Wallis test. In cases of statistical significance, the ranked parameters were compared using the Mann–Whitney U-test and Bonferroni's multiple comparison posttest; a p value < 0.0083 was considered significant.

RESULTS

Skin Flap Survival

Gross examinations indicated that the surfaces of the flaps had healed within 7 days, without complications, in both the experimental and control groups. On postoperative day 7, the surviving regions were clearly distinguishable from the necrotic regions in each flap (Figure 2). The flap area survival rates in the C (21.08 ± 10.3%), LI (57.28 ± 25.8%), LT (60.78 ± 19.1%), and HT (88.03 ± 9.4%) groups were determined. The flap area survival rates were significantly higher in the HPE treatment groups than in the C group ($p < 0.0083$); flap survival in the HT group was also significantly higher than in the LI and LT groups ($p < 0.0083$; Figure 3).

Histopathological assessment

On postoperative day 7, hematoxylin and eosin staining revealed increased numbers of inflammatory cells and marked edema in the C group, compared with the HPE treatment groups (Figure 4). When the pathological scores were compared, the C group had statistically higher scores than the LI group ($p < 0.0083$). In the HT group, the pathology score was significantly lower than that for the LI group ($p < 0.0083$); the LT and HT groups were not statistically different from each other (Table 2).

Immunohistochemical evaluation

Immunohistochemistry revealed the presence of more VEGF-positive microvessels in the HPE-treated groups than in group C (Figure 5). Reflecting this, the VEGF score was significantly higher in the HT group than in the C group ($p < 0.0083$; Table 3 and Figure 6).

Biochemical analysis

1. Glutathione peroxidase

There was a marked improvement in the antioxidant status of the HT group, reflected by the significant increase in GPx concentration, compared with C group ($p < 0.0083$). When mean tissue GPx activities were compared between the C and LT groups, a statistically significant difference was observed ($p < 0.0083$). Additionally, GPx activity was significantly higher in the LT group than in the LI group ($p < 0.0083$). These data indicate that systemically injected HPE protected the flaps from oxidative stress by enhancing GPx activity (Figure 7A).

2. Malondialdehyde

MDA is formed from the breakdown of polyunsaturated fatty acids, and serves as an important and reliable index for determining the extent of peroxidation reactions.[22] Thus, to further examine the antioxidative effects of HPE, we analyzed MDA production in the flap samples. Following random-pattern skin flap surgery, elevated lipid peroxidation resulted

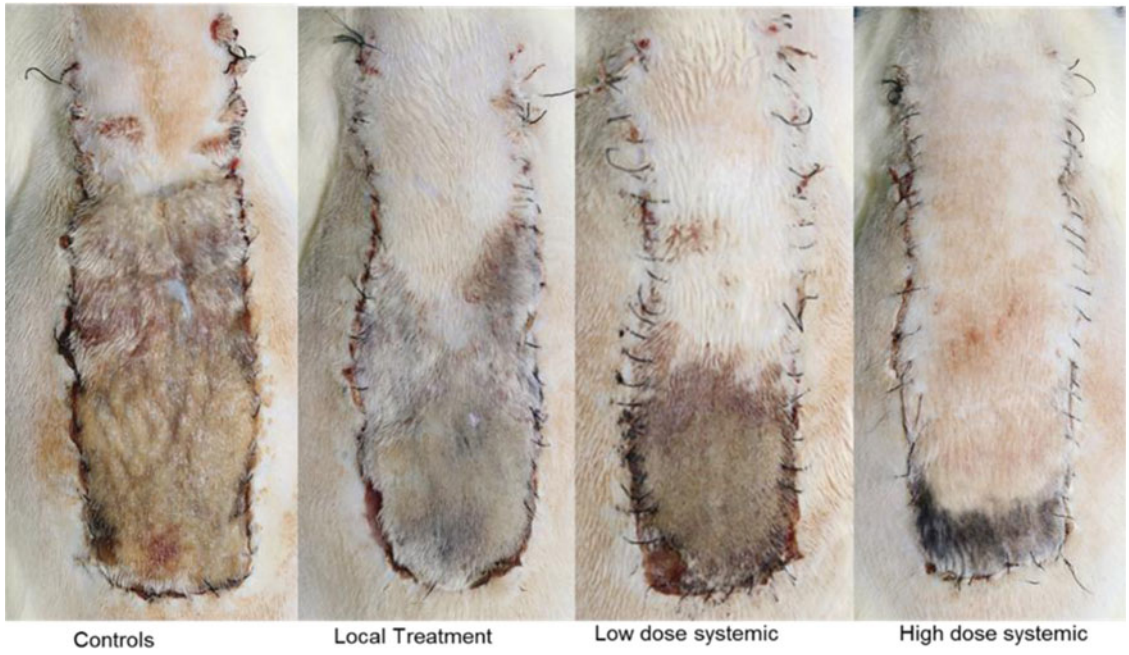

FIGURE 2 Flap appearance on postoperative day 7. The animals receiving high-dose human placental extract (HPE) had the largest average skin flap survival area, followed by those receiving low-dose HPE. Control, left; localized HPE group, middle left; low-dose HPE group, middle right; high-dose HPE group, right.

in increased tissue MDA levels. When mean tissue MDA levels were compared between the control and HPE treatment groups, a statistically significant difference was observed (all, $p < 0.0083$, relative to group C; Figure 7B). The mean tissue MDA level in the HT group decreased by 58.8%, compared with the C group. Moreover, the LT and HT groups demonstrated significantly lower MDA levels than did the LI group (both, $p < 0.0083$). These data showed that both local and systemic HPE injections protected the tissue from increased MDA levels.

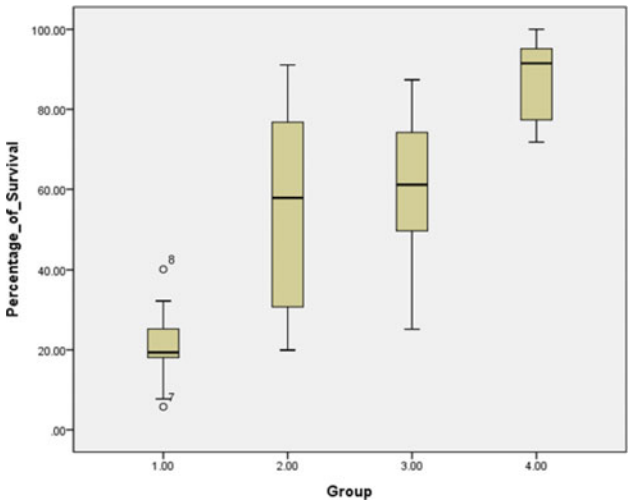

FIGURE 3 Statistical analysis of the flap survival area (%) on postoperative day 7. The necrotic areas were significantly smaller in the human placental extract (HPE) treatment groups, compared with the control group. Moreover, there was significant improvement in random-pattern skin flap survival for the high-dose systemic treatment (HT) group compared with the localized HPE (LI) and low-dose systemic treatment (LT) groups. The mean survival area in the LI group was significantly greater than in the C group ($p < 0.0083$), and that in the HT group was significantly greater than in the LI group ($p < 0.0083$). Group 1, C; Group 2, LI; Group 3, LT; and Group 4, HT.

3. Tissue caspase-3

To determine whether the protective effects of HPE administration correlated with apoptosis inhibition, caspase-3 levels were investigated. There was a statistically significant difference between the control and HPE-treated groups regarding mean caspase-3 activity (all, $p < 0.0083$). When the LT and LI groups were compared with the HT group, statistically significant differences were observed (both, $p < 0.0083$; Figure 7C). The biochemical scores are summarized in Table 4. These data show that both local and systemic treatments prevented increases in caspase-3 activity and effectively inhibited apoptotic cell death due to ischemic injury.

DISCUSSION

HPE, prepared from the placentas of healthy, postpartum females, is known to be physiologically active, including having antioxidative properties.[13, 21, 22] In particular, HPE plays several roles in chronic hepatic disease, and its antioxidant properties have been used to treat oxidative stress-related diseases, such as atherosclerosis and diabetes.[25, 26] The therapeutic ben-

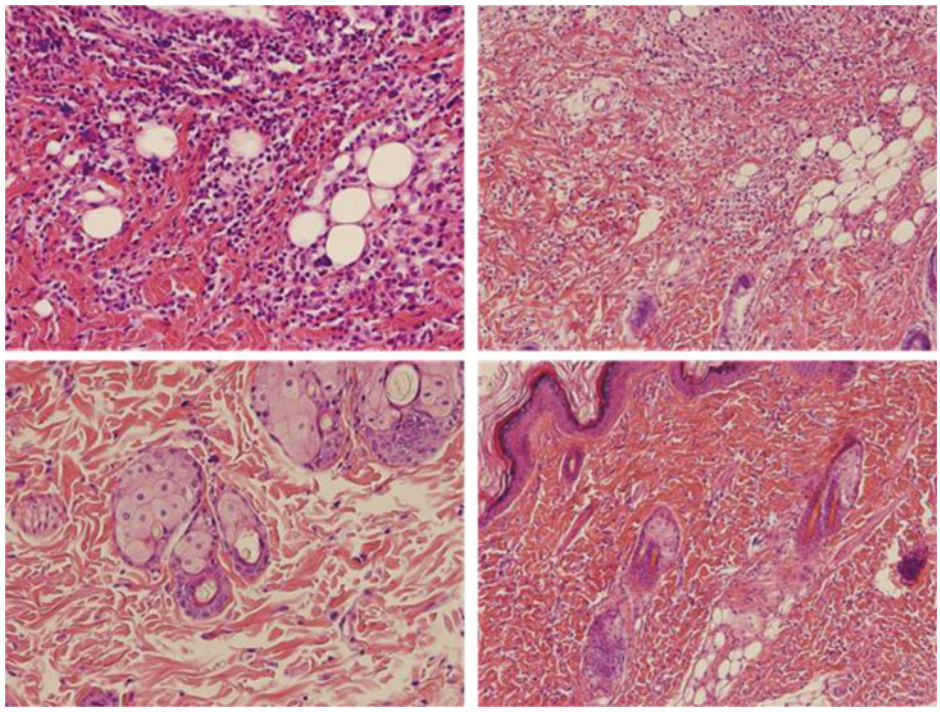

FIGURE 4 Histopathological assessment of tissue from each group (hematoxylin and eosin, ×200). More inflammatory cells are evident in the C group (top left) than in the human placental extract-treated groups. Only minimal inflammatory cell infiltration and interstitial edema is evident in the high-dose systemic treatment (HT) group (bottom right). Statistically significant differences were seen between the localized injection group (top right) and the low-dose systemic treatment (bottom left) and high-dose systemic treatment groups ($p < 0.0083$).

efits of HPE have also been described in the treatment of patients with wounds [27], leading to our hypothesis that HPE would enhance random-pattern skin flap viability by attenuating oxidative stress, suppressing inflammation, and promoting vascularization.

A number of changes detrimental to skin survival occur when a random flap is created. The primary insult affecting flap survival is impaired vascular supply and the resultant ischemia. Tissues receiving insufficient oxygen begin anaerobic metabolism, which also results in elevated free radical activity that leads to activation of proinflammatory enzymes and inflammatory cytokines.[28] The toxic superoxides involved in leukocyte adhesion and accumulation ultimately lead to then results in acute local inflammation, vasoconstriction, increased capillary permeability, and endothelial cell edema.[29] Together, these events impair microcirculation. Moreover, free radicals may directly induce cytotoxic effects by participating in reactions that cause peroxidation of cellular and intracellular membranes and intracellular proteins, resulting in irreversible cell injury.[30]

The role of HPE in preventing oxidative stress has been demonstrated in some organ systems. A preclinical study of concanavalin A-induced liver injury.[31] demonstrated that HPE protects hepatocytes from chronic inflammation by suppressing intercellular adhesion molecule-1 and MDA. In addition, HPE increased superoxide dismutase and decreased oxidative MDA and nitric oxide levels, suggesting that HPE protects liver cells from lipid peroxidation-induced injury.[31] Thus, HPE behaves like an antioxidant and also prevents lipid and protein peroxidation.

The present study showed significant flap survival rate improvements, reflected by significant increases in GPx activity, after systemic HPE injection. HPE may

TABLE 2 Histopathological scores of tissue on postoperative day 7.

Groups	Control	Localized HPE	Low-dose HPE	High-dose HPE
PMNL/Lymphocyte	2.6 ± 0.52	1.5 ± 0.53*	0.5 ± 0.52*	0.1 ± 0.31*
Edema	1.7 ± 0.48	1.0 ± 0.0*	0.5 ± 0.53*	0.2 ± 0.42*
Collagen	1.7 ± 0.0	1.9 ± 0.0	2.0 ± 0.48	2.0 ± 0.32
Total	6.3 ± 0.95	4.4 ± 0.52*	2.9 ± 0.74*	2.0 ± 0.67*

HPE, human placental extract; PMNL, polymorphonuclear leukocyte. Values are expressed as means ± SD; *$p < 0.0083$.

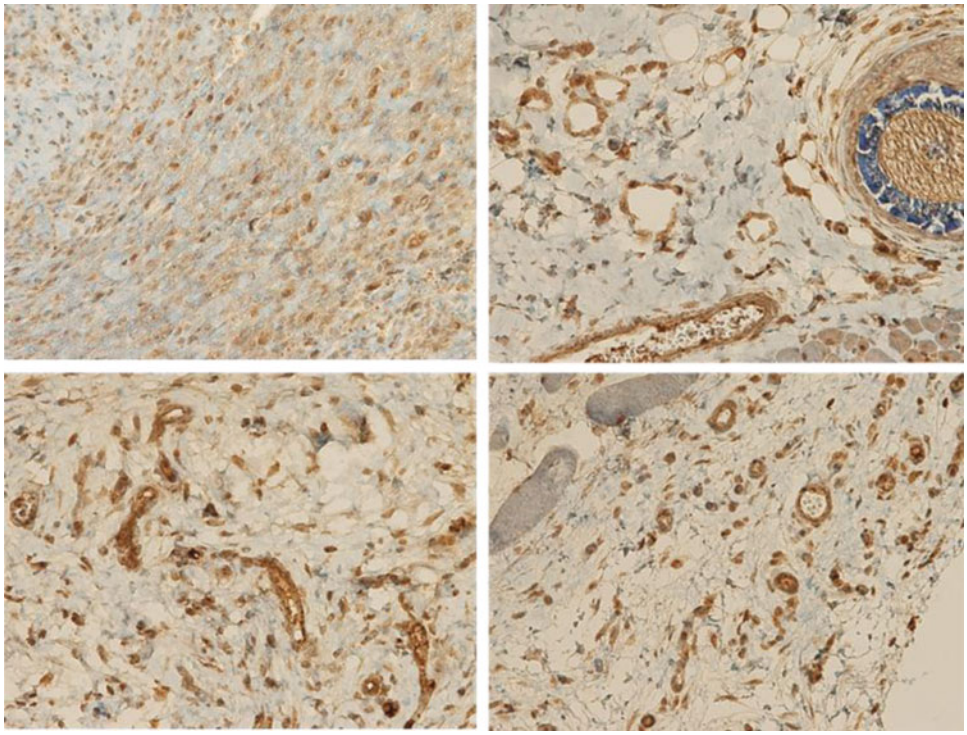

FIGURE 5 Immunohistochemical staining for the presence of vascular endothelial growth factor (VEGF; ×200). Fewer VEGF-positive cells (filled arrows) were seen in the C group (top left) than in the low-dose treatment (bottom left) and high-dose treatment (bottom right) groups. The difference between the C and localized injection (top right) groups was not statistically significant.

stimulate antioxidant enzymes. GPx activity, indicative of glutathione levels and an important antioxidant, showed significant increases in the LT and HT groups, compared with the controls. Further, the increased GPx activity represented the endogenous release of antioxidant enzymes, in response to oxidative stress and high free radical activity in the tissue. Thus, HPE administration may prevent tissue necrosis by controlling free radical levels. The positive antioxidant effect of GPx was also supported by the low MDA levels observed in HPE-treated animals. MDA, a well-known secondary product of cell membrane lipid peroxidation, is formed from the breakdown of polyunsaturated fatty acids, and serves as a convenient index for determining the extent of lipid peroxidation.[32] Our study indicated that HPE administration results in significantly lower tissue MDA levels, possibly decreasing the risk of cellular oxidative injury and preventing lipid peroxidation in the ischemic flap tissue. HPE treatment prevents tissue necrosis by getting rid of the free radical with its antioxidant effect.

Prevention of cell apoptosis has been demonstrated to reduce ischemic damage in many organ systems. Wu et al.[31] analyzed the inhibition of hepatocyte apoptosis following HPE pretreatment by investigating whether the protective effects of HPE pretreatment correlated with apoptosis inhibition, reflected by inhibition of DNA fragmentation and expression of the apoptosis-associated genes bcl-2 and bax. Consistent with these results, we used the caspase-3 assay to examine the antiapoptosis effects of HPE, as reflected by inhibition of caspase-3 activation. Our study indicated that HPE administration results in significantly lower tissue caspase-3 activation, and suggests that the HPE mechanism of action may involve the caspase signaling cascade. HPE protected the flap from apoptosis by lowering caspase-3 activity.

Lee et al.[33] studied the antiinflammatory and analgesic potential of HPE in murine models of inflammation/inflammatory pain. They showed that HPE significantly inhibited the production of nitric oxide, TNF-α, and cyclooxygenase-2. Inflammation plays

TABLE 3 Vascular endothelial growth factor (VEGF) scores and their statistical analysis using the Kruskal–Wallis and post hoc tests.

Groups	Control	Localized HPE	Low-dose HPE	High-dose HPE
VEGF score	7.0 ± 1.94	9.4 ± 2.1	16.1 ± 2.92*	20.6 ± 2.41*

HPE, human placental extract; *$p < 0.0083$.

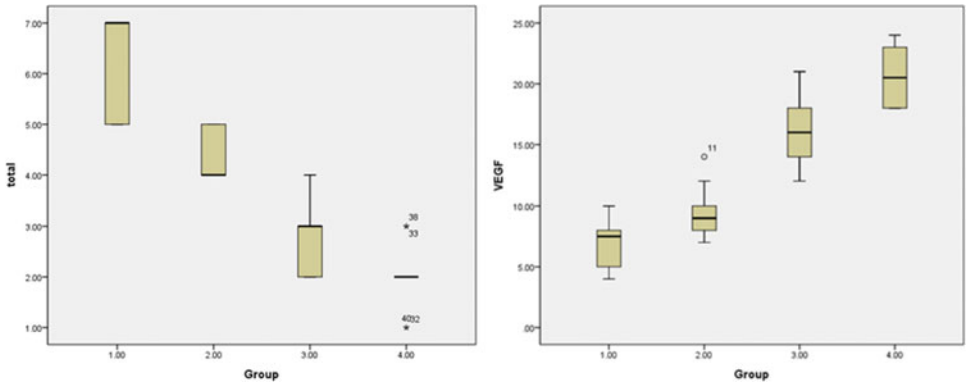

FIGURE 6 Box plots of the histopathologic scores (left) and vascular endothelial growth factor levels (right) on postoperative day 7. Group 1, control; Group 2, localized human placental extract (HPE) injection; Group 3, low-dose systemic HPE; and Group 4, high-dose systemic HPE.

an important role in the survival of random-pattern skin flaps because the extent of inflammation correlates with the extent of necrosis.[34] The antioxidant feature of HPE treatment also was expected to reduce edema caused by free radical. In our study, histopathologic evaluations revealed more tissue edema and inflammatory cells in the tissue samples from group C animals. Interestingly, the HT groups demonstrated significantly ($p < 0.0083$) less edema and reduced polymorphonuclear leukocyte levels than was present in the controls; these observations were linked to the inhibition of free radicals by the HPE treatment. Collagen density was also assessed, but statistically significant differences between the treatment and control groups were not observed.

Angiogenesis, the formation of new capillaries from preexisting venules, is fundamental to wound healing and to vasculature maintenance and repair.[35] However, the potential of growth factors to stimulate angiogenesis is inhibited by free radicals. Thus, antioxidants may play a role in preventing such inhibition and may contribute to the proliferation of endothelial cells. In our study, angiogenesis was immunohistochemically evaluated in tissue biopsy samples stained to detect VEGF, a heparin-binding glycoprotein that is a potent endogenous stimulator of both angiogenesis and vascular permeability. VEGF also promotes endothelial cell growth and migration and inhibits apoptosis, thereby improving flap survival.[36] Relative to skin flap survival, VEGF can stimulate acute vasodilatation, partly through stimulation of nitric oxide synthase in endothelial cells and increasing capillary permeability, in the acute phase.[37] Experimental studies have demonstrated that VEGF acts directly within ischemic flaps, as well as in the surrounding tissues, to promote the growth and development of new vascular channels and improve blood supply through acceleration of arterial blood flow.[38] Hong et al.[39] studied the effect of HPE in a wound-healing model. They showed that HPE significantly accelerates the expression of VEGF, which increased new blood vessel formation in the later stage of wound healing. In this study, significantly more VEGF-positive vessels were observed in the HT group animals than in the controls ($p < 0.0083$). The elevated levels of VEGF in the transplanted flaps may have contributed to the improved flap survival;

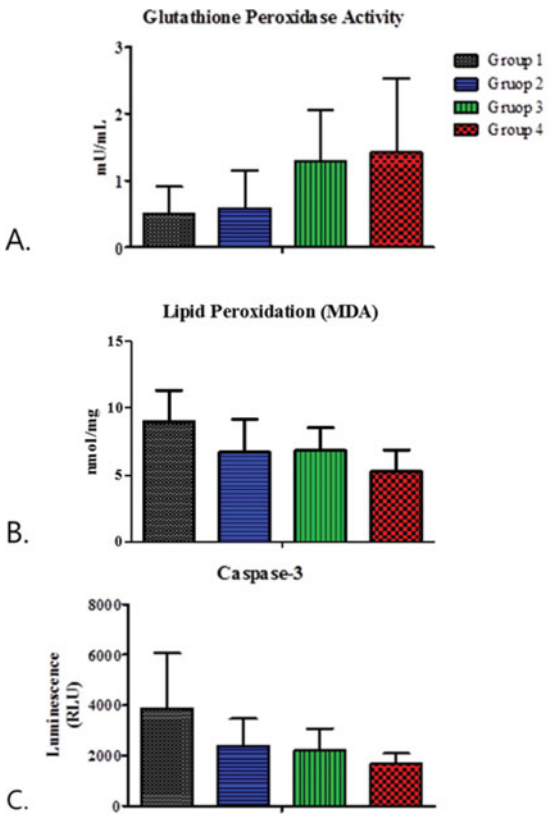

FIGURE 7 Bar graphs showing the biochemical results of tissue glutathione peroxidase (A), malondialdehyde (B), and caspase-3 (C) assays. Values are expressed as means ± SD. Group 1, controls; Group 2, localized human placental extract (HPE); Group 3, low-dose, systemic HPE; and Group 4, high-dose, systemic HPE. RLU, relative light units.

TABLE 4 Results of the biochemical assays for glutathione peroxidase, malondialdehyde, and caspase-3.

Groups	Control	Localized HPE	Low-dose HPE	High-dose HPE
Glutathione peroxidase (mU/mL)	0.5 ± 0.41	0.59 ± 0.56	1.29 ± 0.77*	1.43 ± 1.10*
Malondialdehyde (nmol/mg)	8.98 ± 2.34	6.72 ± 2.42*	6.84 ± 1.71*	5.28 ± 1.61*
Caspase-3 (RLU)	3873.90 ± 2188.46	2390.70 ± 1069.23*	2217.78 ± 851.30*	1675.80 ± 414.81*

HPE, human placental extract; RLU, relative light unit. *$p < 0.0083$.

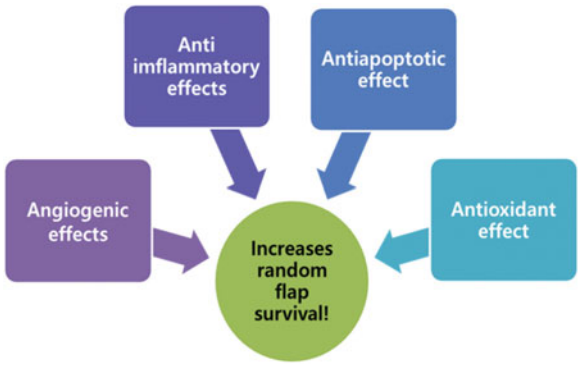

FIGURE 8 Mechanism of HPE treatment on random-pattern skin flap survival.

however, a detailed understanding of the underlying mechanism will require further study.

In this study, we have confirmed that high HPE administration increases random-pattern skin flap survival in rats, which increases the possibility of its wider clinical use. However, to clarify the correlation between medical dosage, application method, and application frequency additional studies are required. Although the HT group animals demonstrated significantly higher GPx activity, reduced necrosis, and reduced MDA and caspase-3 levels than were observed in the other groups ($p < 0.0083$), in animals treated with localized HPE injections, significant differences in GPx activity and VEGF levels were not observed, relative to the control animals. And the failure of localized HPE administration to significantly increase GPx levels or to increase vascularization suggests the need for further investigation. However, this study has some limitations. We do not know the exact components of HPE or their relative amounts.[40] Therefore, further study is also required to identify the active substances involved in ischemic skin flap survival.

CONCLUSIONS

This study demonstrated the beneficial effects of HPE administration on random-pattern skin flap survival. Biochemical and histopathological assessment revealed that HPE exhibits meaningful protective effects against ischemic injury by antioxidant effects, reducing lipid peroxidation, antiapoptotic effects, and angiogenic effects (Figure 8). We also showed that HPE induced dose-related protective effects. In clinical flap surgeries, systemically administered HPE may constitute a novel therapeutic approach to scavenging reactive oxygen species and to inducing an antioxidative response. And we suppose the effect of HPE therapy may vary by depending on the amount of HPE and the routes of HPE delivery. So further study is warranted for that, and optimization of the treatment protocol for clinical use is also needed.

DECLARATION OF INTEREST

None of the authors has a financial interest in any of the products, devices, or drugs mentioned in this article.

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