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The Protective Effect of Prostaglandin E1 Against Ischemia-reperfusion Injury of Musculocutaneous Flaps

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— **Abstract** —

백서 복직근피판에 있어 허혈-재혈류 손상에 미치는 Prostaglandin E1의 예방효과

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본 연구는 백서 복직근피판에 있어 허혈-재혈류 손상에 미치는 prostaglandin E1(PGE-1)의 예방효과를 분석 실험하였으며, 그 기전으로 내피세포의 intercellular adhesion molecule-1(ICAM-1)이 down regulation 됨을 확인하였다. 기존의 PGE-1은 혈관 확장 및 혈소판 응고 저하 등의 기전으로 피판 이식술 후 주로사용하였으나, 허혈-재혈류 손상 시에 PGE-1 역할에 대한 연구는 잘알려진바 없다. 허혈-재혈류 손상에 대한 기전은 현재 여러 가설로 설명되고 있으나, 최근 내피 세포와 백혈구의 역할이 주목을 받고 있다. 장시간 허혈 상태의 피판은 재혈류시 백혈구가 내피세포에 접착함으로써 직간접적인 경로로 독소를생성하며, 결국내피세포및 주변조직의괴사로이어진다. 본연구는면역조직학 염색을 통한내피세포의 ICAM-1 발현 억제와 그로 인한 백혈구의 내피세포 접착 억제를 그 기전으로 볼 수 있었으며, PGE-1을술중투여함으로써피판의생존율을향상시킬 수있었다.

Key Words : Prostaglandin E1, Ischemia-reperfusion injury

Introduction

The effect of prostaglandin E1 on muscle and skin flaps is known[9,17,21]. In treatment for flaps after micro-anastomosis, it can be used on postoperative basis to increase vascular flow by vasodilation and platelet disaggregation. But latest researches have demonstrated its protective efficacy on liver ischemia-reperfusion injury[11,18,20]. Reperfusion injury was first described by Ames et al

which stated that obstruction of the microcirculation and necrosis of the reperfused tissues follows after prolong period of ischemia[1]. The pathophysiology of ischemic-reperfusion injury is complex and includes oxygen radicals, platelet aggregation, and leukocytes-endothelial interactions. Recently, greater attention has been focused to the interaction between neutrophils and endothelial cells as a mechanism of reperfusion induced injury[4,13]. Neutrophils, after binding to the endothelial cells, are known to release cytokines, enzymes, oxygen free radicals and with their viscid properties obstruct the capillaries thus impairing oxygen supply to the tissue. For the neutrophils to interact with endothelial cells, inter-cellular adhesion molecule (ICAM-1) needs to be expressed on the membranes of the endothelium. ICAM-1 is a ligand on the endothelium for some of the CD11a/CD18 and CD11b/CD18 adhesion receptors, lymphocyte function associated antigen-1(LFA-1), on the leukocytes and enables activated leukocytes to adhere firmly within the microcirculation(Fig. 1). Many researchers have reported that ICAM-1 expression increases upon reperfusion after ischemic insult, rapidly during the first 8 hours followed by slow elevation over period of days. Its expression remained elevated for 7 days[2,18,19,23]. Latest researches show that prostaglandin E1 may have a down regulating effect for the ICAM-1 thus reducing neutrophil-endothelial interaction of reperfused tissues[18].

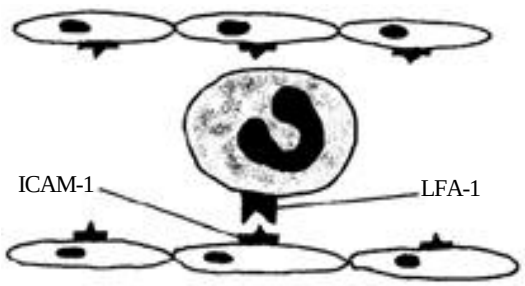

Fig. 1. Circulating polymorphonuclear cell (PMN) attaching to endothelial cells through intercellular adhesion molecule-1 (ICAM-1) and lymphocyte function associated (LFA-1) molecule on the PMN surface.

In this study, the attempts are made to demonstrate the effect of prostaglandin E1 in reperfusion induced flap injury.

Material & Method

1. Surgical Procedure

Thirty-five Sprague-Dawley rats weighing 250~350 grams were used. All procedures were performed aseptically under general anesthesia using intramuscular injections of ketamine hydrochloride(Ketalar®, Yuhan corporation, Seoul) with dosage of 4㎎/kg. The rats received antibiotic therapy using intramuscular injection of gentamycin 4㎎/kg postoperatively. The rats were bred in the same standard environment.

After shaving and washing the abdomen, single superior epigastric vessel pedicled transverse rectus abdominis musculocutaneous (TRAM) flaps were designed and elevated at the dorsum of the rat with size of 6×5㎝. Skeletonization of a single superior epigastric artery and vein pedicle was achieved and made ready for ischemia. The contra-lateral superior epigastric vessel and both inferior epigastric vessels were suture-ligated. Immediately after clamping, the second group was injected with prosta-glandin E1 within the muscle flap whereas the first group was injected with the same amount of normal saline. Injection of prostaglandin E1 (Prostandin®, Dong-A pharmaceutical, Seoul) was made consistently at three sites, proximal, middle and distal portion of the

rectus muscle, with total dosage of 1μg mixed to 0.6cc of injectable saline. Ischemic time of 2 hours followed by reperfusion and reposition of the flap was performed using non-absorbable suture material. The reperfusion was confirmed by bleeding from the distal epigastric artery and then ligated. The flaps were repositioned above an aseptic latex glove to prevent vascular supply other than the pedicle with continuous 5~0 monofila-ment nylon sutures. The rats were euthanized after 24 hours and on the fifith day for analysis. The rats were divided into three groups; group 0: sham operated group(n=5), group 1: the control group with ischemic insult and injection of normal saline 0.6cc within the muscle flap(n=15), group 2: prostaglandin E1 intra-flap injection of 1μg immediately after ischemic insult for 2 hours followed by reperfusion(n=15). The analysis consisted of gross flap measurements, monoclonal antibody and histologic analysis.

2. Flap measurements

The flaps were evaluated 5 days after reperfusion(n=5 for group 0, n=10 for group 1 and 2). Necrotic area of the cutaneous island of the flap was determined by color, refill, eschar and pin-prick test. The outlines of viable and non-viable areas were traced using a transparency film. Then the film was scanned and using an image analysis program(soft imaging system analysis® 3.0), the viability of the flap was calculated. Viability of cutaneous island of the flap=(viable area/total area of cutaneous island)×100%. Statistical analysis was made using student t-test.

3. Monoclonal Antibody Analysis

The flap was evaluated 24 hours(n=5 for group 1 and 2) and 5 days after reperfusion (n=10 for group 1 and 2). For each flap, three 4mm sectioned tissue blocks were preserved in 10% formalin taken from the margin between the viable and necrotic flap, viable flap, and necrotic portion of the flap. A 4-㎛-thick sections were made and embedded in paraffin for immunohistologic analysis. 1A29, a monoclonal antibody to rat ICAM-1(anti-rat CD54; mouse IgG_1) from BD PharMingen Inc., was used for immunohistochemical staining. Histopathologists blinded to the two groups analyzed the immunohistochemical staining.

4. Histological Analysis

The flap was evaluated 24 hours(n=5 for

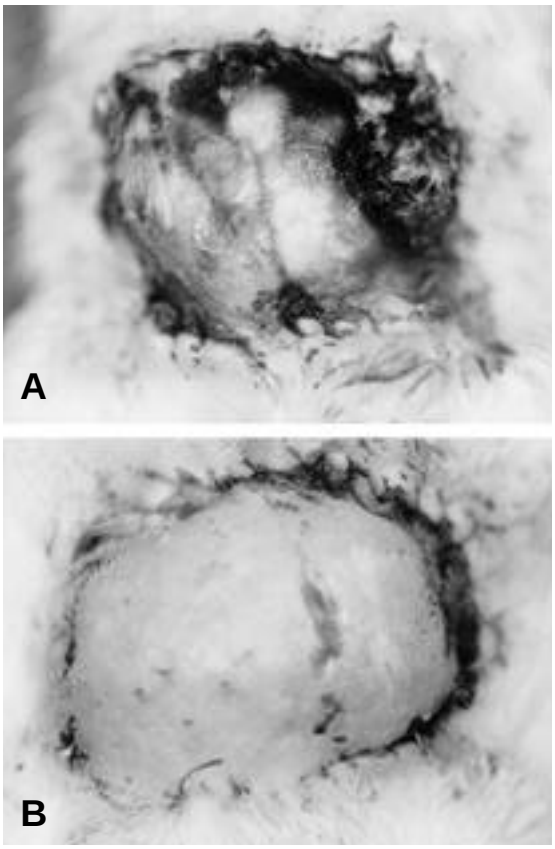

Fig. 2. Note the extensive necrosis of the control group (A) Typical outcome of the prostaglandin E1 treated group (B).

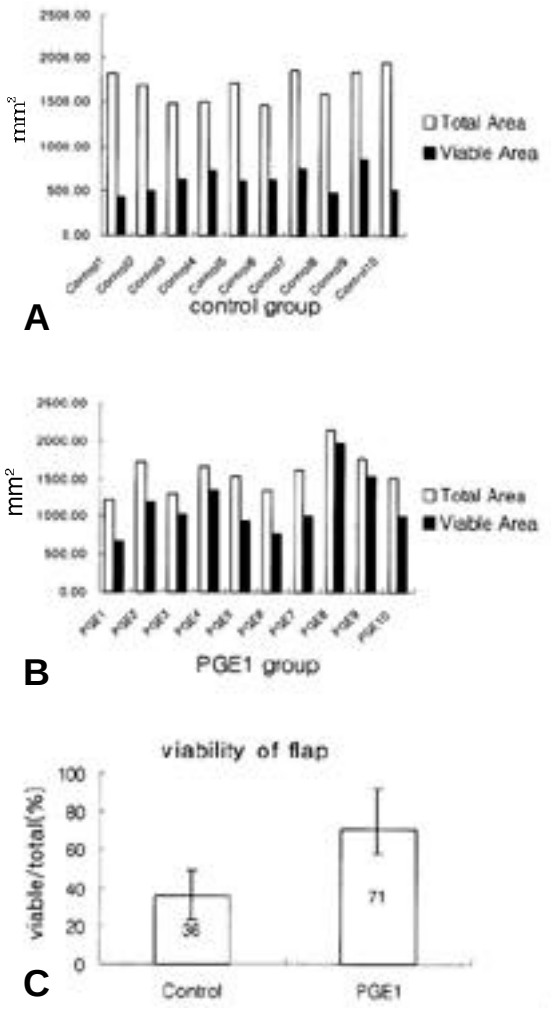

Fig. 3. The viable areas are shown in the bar graph for each group (A,B). The viable portion of the flap is compared between the two groups showing increased viable area in the prostaglandin E1 group ($p<0.01$)(C).

group 1 and 2) and 5 days after reperfusion (n=10 for group 1 and 2). For each flap, three 4mm sectioned tissue blocks were fixed in 10% formalin and embedded in paraffin for hematotoxylin and eosin(H & E) stain taken from the margin between the viable and necrotic flap, viable flap, and necrotic portion of the flap. Histologic analysis is made comparing the edema, inflammatory reactions, and vascular structure. Sticking leukocytes were counted within 3 randomly selected vessels of sizes ranging from 30 to 50㎛. These sections were reviewed by a histopathologists blinded to the two groups to determine extent of injury. Statistical analysis was made using student t-test.

Results

1. Flap Measurements

After measuring each flap, the group 2 treated with prostaglandin E1 had an average viable area of 71 percent compared to control group which had an average of 36 percent. The sham operated group showed 100% survival of the flap. The treatment of prostaglandin E1 significantly reduced the flap necrosis after reperfusion($p<0.01$)(Fig. 2,3). The extent of muscle necrosis was relatively correlated to the area of skin necrosis.

2. Monoclonal Antibody Analysis

Examination of ICAM expression on endothelial cells after 24 hours showed that immunohistochemistry for ICAM-1 of TRAM flap revealed stronger expression in the control group compared to the ones treated with prostaglandin E1. The ICAM-1 expression of group 1 was noted 5 days after reperfusion. Under high power magnification, expressions were minimal or nearly nill for the prostaglandin E1 group(Fig. 4).

3. Histologic Analysis

The degree of inflammation, muscle cell death, and leukocyte adhesion on endothelium was compared between group 1 and 2. In the control group, abundant inflammatory changes was observed whereas the prostaglandin E1 treated group showed less edema and inflammation in regions of the flap after 24 hours. The findings after 5

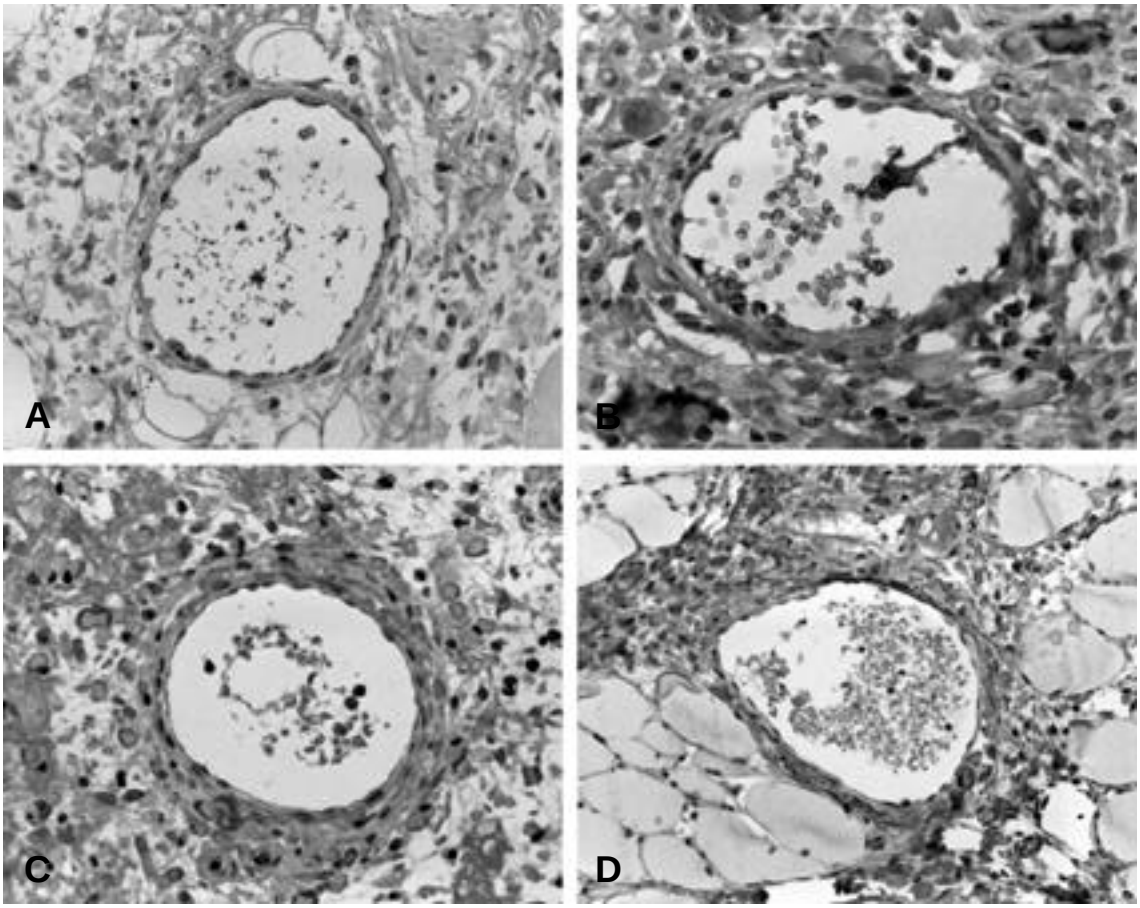

Fig. 4. Immunohistochemical staining for ICAM-1 expression on the endothelium under ×200 magnification. Note the increased expression on the endothelium of the control group (A, B) and minimal expression of the prostaglandin E1 treated group (C, D).

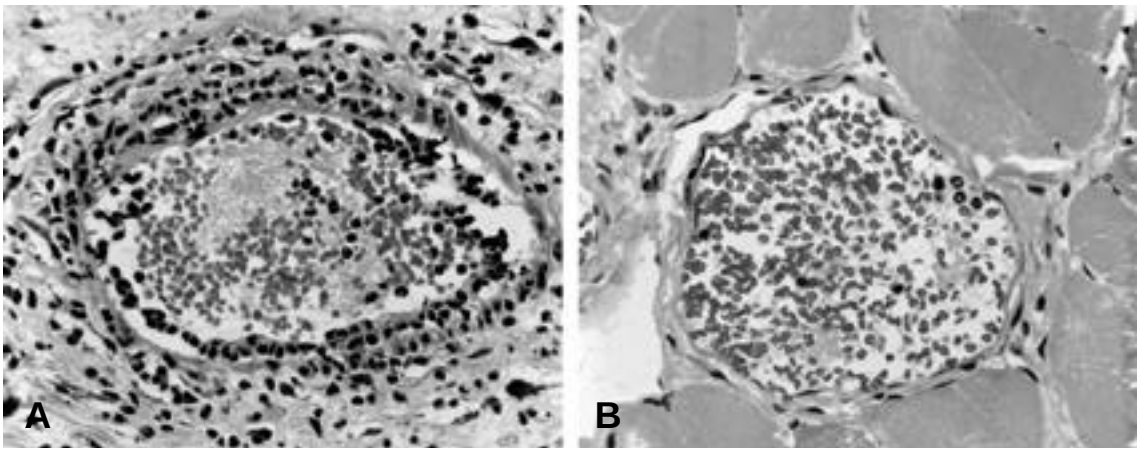

Fig. 5. Increased adhesion of the leukocytes on the endothelium is noted in the control group (A) and minimal adhesion of group treated with prostaglandin E1 (B). (hematotoxylin and eosin stain, ×400).

days revealed further damage to the tissues in group 1. Interstitial inflammation was especially overwhelming in the control group. Most of the inflammation consisted of plasma cells, histiocytes, lymphocytes, and neutrophils. The neutrophils predominated the control group. The control group also showed extensive muscle cell death with clumping of nuclei, necrosis and entire replacement of muscle with inflammation whereas the group treated with prostaglandin E1 revealed mild to moderate muscle cell death with or without hypereosinophilia. Also the prostaglandin E1 group showed evidence of abundant neovascularization and granulation. The regions of necrosis showed no significant differences among the two. The numbers of sticking neutrophils showed significant increase in the control group(Fig. 5). The total numbers of neutrophil adhesive to the endothelium, in three selected vessels with sizes of 30 to 50㎛., was uncountable in group 1 whereas group 2 ranged from 14 to 4(average: 9.4) after 24 hours. The neutrophil adhesion 5days after reperfusion ranged from 52 to 21(average: 33.2) in the control group whereas the prostaglandin E1 group ranged from 8 to 1(average: 3.9)(Fig. 6). The leukocyte adhesion in the control group was statistically significant($p<0.01$).

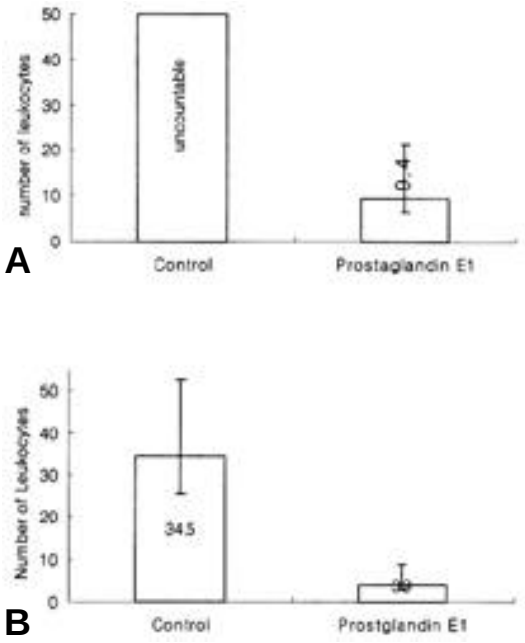

Fig. 6. Adhesion of leukocytes on the endothelium in two groups 24 hours (A) and 5 days (B) after reperfusion. Three selected vessels with size of 30~50 ㎛. was counted. Significant increase of leukocyte adhesion to endothelium I shown in the control group ($p<0.01$).

Discussion

Many researchers have represented the accumulating evidence that polymorphonuclear neutrophils interact with vascular endothelial cells during reperfusion, resulting in leukocyte adhesion and the release of various inflammatory mediators[2,4,7,13,15,19,24,26]. The neutrophil is activated after tight adhesion to the endothelium[16]. The release of oxygen free radicals and elastase by activated neutrophils can damage the endothelial cells directly and by triggering lipid peroxidation and formation of hydroxyl radicals within the cells causes indirect injury[10,13,25]. These multiple cascade of neutrophil reactions can lead to a devastating common final pathway, obstruction of blood flow known as ischemia-reperfusion injury. In this study, the control group with extensive flap necrosis showed statistically significant increase in number of sticking leukocytes to endothelial cells. The surrounding tissue revealed extensive inflammatory response in the control group whereas the treatment group showed much less inflammation. These fact supports that leukocyte does participate in the role of inducing ischemia-reperfusion injury leading to flap necrosis. The function of prostaglandin E1, when treating flaps, is

vasodilation and platelet disaggregation that leads to increase vascular flow. Recently, several studies have shown that prostaglandin E1 also works as a cytoprotective agent against ischemia-reperfusion injury[11,18,20]. But their use as a protective agent against ischemia-reperfusion injury of flaps is undetermined and lacks in-depth investigation.

The method of intra-flap injection was chosen to minimize the systemic effect of prostaglandin. Kaley and Weiner[12] demonstrated the effect of prostaglandin E1 when locally injected and revealed its vasodilating effect on peripheral vessels. To down-regulate the ICAM-1 expression on the endothelium prior to reperfusion was the basis for intra-op injection immediately after clamping. The effect of preoperative injection of prostaglandin E1 is currently under research.

The transverse rectus abdominis musculocutaneous(TRAM) flap is a well-established flap in rats. Dunn et al[6] when based on a single superior epigastric artery demonstrated consistency and reproducibility as a myocutaneous flap. The pilot study of the flaps after two hours of warm ischemia produced significant necrosis of the skin island and relatively reproducible survival of 30 to 50% of cutaneous island in the control group was obtained. The necrosis began from the contra-lateral and the distal portion of the flap. The use of prostaglandin E1 increased the survival area of the cutaneous island to a significant 71%. This result demonstrates increased tolerance to ischemia resulting in increased survival in the treated group. This finding in combination of results obtained in the histologic evaluation of leukocyte adhesion and immunohistochemical analysis of ICAM-1 expression can suggest the role of prostaglandin E1 in ischemia-reperfusion injury. Adhesion molecules are essential for neutrophils to adhere tightly to the endothelium. Lymphocyte function associated-1(LFA-1) molecules are expressed on the surface of the neutrophils and remained unchanged after reperfusion. They adhere tightly to ICAM-1 on the surface of the endothelium. The neutrophil-endothelial interaction is divided to early and late adhesion[13,26]. The early endothelial cell dependent adhesion is associated with P-electin(GMP-140, a member of the selectin family of adhesion molecule) during the first 30 to 45 minutes after reperfusion. The late response consists of E-selectin(ELAM-1, a member of the selectin family of adhesion molecule) and ICAM-1. ICAM-1 demonstrates slower induction, protracted responsiveness, and may play a role in chronic rather than acute inflammatory reactions[13,26]. These molecules can be targeted to limit or prevent ischemia injuries caused by leukocytes. Pretreatment with monoclonal antibodies against CD-18(LFA-1) has demonstrated to reduce neutrophil adherence to endothelial cells[15,18]. Also, research has shown that a monoclonal antibody to ICAM-1 can alleviate the severity of injury of skin flaps subjected to periods of warm ischemia[22]. By directly blocking the adhesion of leukocytes to endothelial cells or by down-regulating the receptors in which the leukocyte adheres to, either way can effectively reduce interaction between leukocyte and endothelial cells involved in ischemia-reperfusion injury. The actual adhesion of leukocytes was significantly reduced in the group treated with prostaglandin E1 that decreased the expression of ICAM-1. Other effects of

prostaglandin E1 such as relaxation of smooth muscle of vessels, increase in fluidity of erythrocytes, and increasing the activity of cyclic AMP and suppression of calcium ion influx of platelets leading to platelet disaggregation all directs toward better survival of flaps[3,5,8]. The suppression of tumor necrosis factor production from macrophages, inhibition of T-lymphocyte cytotoxicity and mitogenesis has also been reported[14]. Along with these numerous cytoprotective functions of prostaglandin E1, better survival of flap was achieved. This study demonstrates that prostaglandin E1 attenuated neutrophil adherence to endothelium exposed to ischemia-reperfusion in rats during the early and late inflammatory phase. Fluorescence microscopy revealed the decreased expression of ICAM-1 in the group treated with prostaglandin E1. By these observations, prostaglandin E1 can be assumed to limit leukocyte adhesion to endothelium through suppression of expression of ICAM-1 in endothelial cells. The exact mechanism on how down regulation of the expression ICAM-1 on the surface of the endothelial cells results still needs to be researched.

Conclusion

This study shows the correlation of leukocytes to ischmia-reperfusion injury and that prostaglandin E1 exerts a protective effect on limiting the ischemic injury of myocutaneous flaps. The prostaglandin E1 exerts its function by down-regulating the expression of ICAM-1 on the endothelial cells decreasing the adhesion of leukocytes during the early and late phase of inflammatory response. These data supports the role of prostaglandin E1 in ischemia-reperfusion injury and flap survival.

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