Prostaglandin E₂ EP4 receptor–selective agonist facilitates sternal healing after harvesting bilateral internal thoracic arteries in diabetic rats

Akira Marui, MD, PhD,^a Keiichi Hirose, MD,^a Takayuki Maruyama, PhD,^b Yoshio Arai, MD,^a Yuhong Huang, MD,^a Kazuhiko Doi, MD,^c Tadashi Ikeda, MD, PhD,^a and Masashi Komeda, MD, PhD^a

Objective: Sternal wound complications are devastating events occurring in coronary artery bypass surgery, particularly in patients with diabetes. Prostaglandin E₂ receptors have 4 subtypes, and the activation of the EP4 receptor induces bone regeneration. The present study investigated the utility of a prostaglandin E₂ EP4 receptor–selective agonist in sternal healing after median sternotomy with the removal of the bilateral internal thoracic arteries in diabetic rats.

Methods: Diabetic Wistar rats with blood glucose levels of greater than 400 mg/dL were established by means of a single intraperitoneal injection of streptozotocin. After median sternotomy and bilateral internal thoracic artery removal in 16 diabetic rats, 8 rats were administered the EP4 agonist (300 μ g) on the posterior table of the sternum (EP4 group), whereas 8 did not receive any treatment (control group). Sternal healing and incidence of sternal wound complications were evaluated 4 weeks after the operation.

Results: Sternal wound complications developed in 5 rats in the control group but in only 1 rat in the EP4 group ($P < .01$). Histologic examination revealed an almost completely healed sternum filled with regenerated bone tissue only in the EP4 group. Both bone mineral content and bone mineral density, as assessed with dual-energy x-ray absorptiometry, were higher in the EP4 group than in the control group (71.7 ± 12.1 vs 48.9 ± 11.7 mg for bone mineral content [$P < .01$] and 66.8 ± 14.6 vs 47.9 ± 6.3 mg/mm² for bone mineral density [$P < .05$]).

Conclusions: The prostaglandin E₂ EP4 agonist accelerated the sternal healing and decreased the incidence of sternal wound complications in the diabetic ischemic sternum. This method might help in decreasing sternal necrosis in high-risk patients or permit wider application of bilateral internal thoracic arteries in coronary artery bypass surgery, even in patients with diabetes.

From the Department of Cardiovascular Surgery, Kyoto University Graduate School of Medicine,^a Kyoto Japan; Minase Research Institute, Ono Pharmaceutical Company,^b Osaka, Japan; and Takeda Hospital,^c Kyoto, Japan.

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Address for reprints: Masashi Komeda, MD, PhD, Professor and Chairman, Department of Cardiovascular Surgery, Kyoto University Graduate School of Medicine, 54 Shogoin-Kawara, Sakyo, Kyoto, 606-8507 Japan (E-mail: komelab@kuhp.kyoto-u.ac.jp).

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Sternal wound complications (SWCs; ie, sternal dehiscence [instability], infection after median sternotomy, or both) are potentially devastating events occurring after cardiac surgery.^{1,2} Bilateral internal thoracic artery (BITA) grafting after median sternotomy in coronary artery bypass surgery carries a high risk of SWCs, particularly in patients with diabetes mellitus.³⁻⁶ Therefore, the use of BITA grafts in patients with severe diabetes remains controversial, although some institutions have little concern regarding SWCs.^{7,8}

Sternal ischemia is an important risk factor for SWCs. In addition, delayed sternal healing followed by sternal dehiscence is another important risk factor for SWCs.¹⁻⁶ Therefore both sternal blood perfusion recovery (angiogenesis) and the acceleration of sternal healing (osteogenesis) might be important for the prevention of SWCs.

We have previously reported that topical use of a gelatin hydrogel sheet incorporated with basic fibroblast growth factor (bFGF), a potent angiogenic and osteo-

Abbreviations and Acronyms

bFGF	= basic fibroblast growth factor
BITA	= bilateral internal thoracic artery
BMC	= bone mineral content
BMD	= bone mineral density
DEXA	= dual-energy x-ray absorptiometry
PG	= prostaglandin
PLGA	= copoly lactic acid/glycolic acid
SWC	= sternal wound complication

genic factor, ameliorated sternal ischemia and prevented SWCs.^{9,10} However, in these series bFGF alone could not completely prevent SWCs, and this might be attributed to the insufficient osteogenic effects of bFGF compared with its strong angiogenic effects.

Prostaglandins (PGs) are a group of lipid mediators produced from arachidonic acid and are found in various tissues.^{11,12} PGE₂ stimulates bone formation and increase the bone mass in vivo. Four types of PGE₂ receptors (ie, EP1, EP2, EP3, and EP4) have been identified, and EP4 is the major receptor that mediates bone formation.¹¹⁻¹⁵

We have developed a PGE₂ EP4 receptor-selective agonist that induces bone regeneration.¹⁵ In the present study we tested the hypothesis that the EP4 receptor-selective agonist accelerates sternal healing and decreases the incidence of SWCs after median sternotomy with BITA removal in streptozotocin-induced diabetic rats.

Materials and Methods**Chemicals**

An EP4 receptor-selective agonist, ONO-4819 (methyl 7-[(1R, 2R, 3R)-3-hydroxy-2-[(E)-(3S)-3-hydroxy-4-(methoxymethylphenyl)-1-butenyl]-5-oxocyclopentyl]-5-thiaheptanoate; Patent Cooperation Treaty publication no. WO 00/03980), shows inhibition constant values of 0.7, 56, and 620 nmol/L for radioligand binding to EP4, EP3, and EP2, respectively, and values of more than 10 μ mol/L for EP1 and receptors for PGD₂, PGF_{2 α} , PGI₂, or thromboxane A₂.¹⁵

Preparation of Copoly Lactic Acid/Glycolic Acid Microspheres Containing the PGE₂ EP4 Agonist

To have effective bone-forming activities, ONO-4819 needs repeated injection because of its short half-life.¹⁵ We used copoly lactic acid/glycolic acid (PLGA) as a slow-release carrier for the agonist. To obtain desirable release profile of the agonist from the PLGA, ONO-AE2-724 was produced as a prodrug of ONO-4819 by introducing an acyl chain (C9) to its carboxylic part.

ONO-AE2-724 (10 mg) and PLGA75-65 (90 mg) were dissolved in 1 mL of dichloromethane as the oil phase. The oil phase was gradually added into aqueous polyvinylalcohol (0.1%) solution under stirring with a turbine-shaped mixer (Homomixer) at 6000 rpm to obtain oil-in-water emulsion. Then the PLGA microspheres were suspended in the polyvinylalcohol solution after organic solvent evaporation. Supernatant was discarded and re-

placed with fresh water or aqueous medium containing 0.2% Tween 80. Washed microsphere precipitation was lyophilized to remove residual organic solvent and water, and then dried solid ONO-AE2-724 microspheres were recovered. ONO-AE2-724 was slowly released from the PLGA microspheres approximately for 4 weeks.

Preparation of Fibrin Glue as a Scaffold of the PLGA Microspheres Containing the EP4 Agonist

We used fibrin glue as a scaffold of the PLGA microspheres containing the EP4 agonist. Tisseel (1.0-mL kit, Baxter) was prepared according to the manufacturer's instructions. Briefly, the contents of the thrombin vial were resuspended with 0.5 mL of CaCl₂ solution, and the sealer proteins in the second vial were reconstituted with 0.5 mL of aprotinin solution. Both vials were warmed at 37°C for 5 minutes, with constant stirring to ensure complete dissolution. The PLGA microspheres incorporating the EP4 agonist were dispersed to the thrombin solution. When the operation was near completion, the contents of each vial were drawn into separate syringes and attached to the applicator device, and 200 μ L of fibrin glue was delivered to the posterior table of the sternum.

Animals

Preparation of rats with sternal ischemia after median sternotomy. Rats with sternal ischemia after median sternotomy were prepared as previously described.^{9,10} In brief, median sternotomy was performed by using a rotating saw, and the BITAs were ligated with 6-0 polypropylene sutures near the origin and at the distal bifurcation. Then the BITAs, with their beds, were destroyed by use of an electrical coagulator.

Induction of diabetes mellitus. Male Wistar rats weighing between 250 and 300 g were used for diabetic models. Diabetic rats were created by means of a single intraperitoneal injection of 55 mg/kg streptozotocin (Wako Chemicals) in 0.1 mol/L citrate buffer (pH 4.9). Diabetes mellitus was defined by both an increase in blood glucose levels of greater than 400 mg/dL and a loss of body weight of greater than 15 g 2 weeks after the injection.

The Kyoto University Animal Experiment Committee approved the experiments. Animals were cared for in compliance with the "Guide for the care and use of laboratory animals," Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council.

Study Group Profiles

Study 1: Dose effects of the EP4 agonist in sternal healing after median sternotomy. To determine the therapeutic dose of the EP4 agonist, we evaluated the dose effects of the EP4 agonist in nondiabetic-nonischemic sternum. Thirty-six nondiabetic rats with median sternotomies were randomly divided into 6 groups (Figure 1): rats in the EP4-treated groups had fibrin glue containing the PLGA microsphere with various doses of the EP4 agonist (1000, 300, and 100 μ g, respectively) on the posterior table of the sternum; rats in the control group had no treatment; and rats in the vehicle group had fibrin glue containing the PLGA microspheres without the EP4 agonist on the posterior table of the sternum. In addition, unmanipulated rats were also evaluated.

Rats were killed by means of intravenous administration of a lethal dose of sodium pentobarbital 4 weeks after the operation.

Figure 1. Dose effects of the EP4 agonist assessed by means of dual-energy x-ray absorptiometry. Dose effects of the EP4 agonist were evaluated in nondiabetic-nonischemic murine sternum after median sternotomy. Bone mineral content (BMC) and bone mineral density (BMD) were measured 4 weeks after the operation: a-c, the EP4-treated groups (rats treated with 1000 µg [a], 300 µg [b], and 100 µg [c] of the EP4 agonist); d, the control group (rats without treatment); e, the vehicle group (rats with vehicle alone); and f, the unmanipulated group. * $P < .01$ and # $P < .05$ vs control; † $P < .05$ vs 100 µg of the EP4 agonist. n.s., Not significant.

The sternum was excised and fixed in 10 wt% formaldehyde solution in phosphate-buffered saline for 4 days to assess the extent of the sternal healing.

Study 2: Effects of the EP4 agonist on diabetic rats with median sternotomies with the BITAs removed. The 16 diabetic rats that had median sternotomies and BITA removal were randomly divided into 2 groups ($n = 8$ each): rats in the EP4 group had fibrin glue containing the PLGA microspheres incorporated with a therapeutic dose of the EP4 agonist on the posterior table of the sternum before closing the sternum, whereas rats in the control group had the fibrin glue containing the PLGA microspheres without the EP4 agonist in the same manner. The sample of the sternum was harvested as described in study 1 for histologic assessment.

Assessment of Sternal Bone Healing

Dual-energy x-ray absorptiometry. Qualitative and quantitative analysis of bone regeneration was assessed on the basis of bone mineral content (BMC) and bone mineral density (BMD). The BMC and BMD of each sternum were measured with dual-energy x-ray absorptiometry (DEXA) by using a bone mineral analyzer (Dichroma Scan 600, Aloka Co) 4 weeks after the operation. The instrument was calibrated with a phantom of known mineral content. Each scan was performed at a speed of 20 mm/s, and the scanning length was 1 mm.

Soft x-ray film analysis. Soft (high-contrast) x-ray films of the sternum were taken at 46 kV and 2 mA for 45 seconds by use of an x-ray apparatus (type CMB, Koizumi X-Senkosha). Films of formalin-fixed bone specimens from different experimental groups were taken with the use of the same type of x-ray film.

Histology. Bone specimens were demineralized in 10 wt% ethylenediamine tetraacetic acid solution at 4°C for 3 days, embedded in paraffin, and sectioned at 10-µm thickness. The sections were obtained at the third, fourth, and fifth intercostal spaces of the sternum and stained with hematoxylin and eosin 4 weeks after the operation. The histologic sections were analyzed by use of a microscope equipped with a video camera connected to an image analysis system (SP-1000, Olympus).

Assessment of Peristernal Blood Perfusion Recovery

Measurement of peristernal blood perfusion. Peristernal blood perfusion was measured with a laser Doppler perfusion image analyzer (Moor Instruments) before the median sternotomy, after closure of the sternum, and 4 weeks after the operation, as previously described.^{16,17} Excess hairs were removed with surgical clippers the day before scanning. Rats were placed on a heating plate at 37°C to minimize temperature variation. The Laser Doppler imaging measured the blood perfusion in the 4-cm length $\times$ 1.5-cm width of bilateral peristernal areas. The average blood flow was calculated for evaluation.

Histology. The peristernal number of vessels was counted, as previously described.⁹ Briefly, arterioles (>25 and <100 µm in external diameter) and capillaries (<25 µm in external diameter) were counted in preparations stained with hematoxylin and eosin in a 200 $\times$ field (0.442 mm² per unit area). Five fields were chosen randomly from the connective tissue around the sternum. Two pathologists blinded to treatment counted the number of vessels per unit area.

Statistical Analysis

Experimental results are expressed as means $\pm$ standard deviation. Multiple group comparisons were performed by using analysis of variance. In multiple comparisons among independent groups in which analysis of variance indicated significant differences, the statistical value was determined according to the Bonferroni-Dunn method. Statistical analysis comparing 2 groups was performed with the Wilcoxon rank sum test for means or the Fisher exact probability test for categorical variables. All statistical analyses were performed with Statview software (Abacus).

Results

Study 1: Dose Effects of the EP4 Agonist

Neither the BMC nor the BMD in the vehicle group differed from those in the control group at 4 weeks after the operation (Figure 1). In contrast, all 3 doses of the EP4 agonist produced a significant increase in both the BMC and BMD. The BMC and BMD of rats treated with 300 µg of the EP4 agonist were significantly higher than those of rats treated with 100 µg (93.6 ± 13.8 vs 71.4 ± 11.7 mg for BMC and 91.1 ± 11.9 vs 73.1 ± 7.3 mg/mm² for BMD; $P < .05$, respectively; Figure 1). However, the effects of 300 µg and 1000 µg of the EP4 agonist were not significantly different from each other (93.6 ± 13.8 vs 98.3 ± 10.5 mg for BMC and 91.1 ± 11.9 vs 95.3 ± 12.3 mg/mm² for BMD, Figure 1). Therefore 300 µg of the EP4 agonist was used as the therapeutic dose in study 2.

Figure 2. Soft x-ray films and the incidence of sternal wound complications (SWCs) 4 weeks after the operation. **A**, Soft x-ray films of SWCs. Sternal dehiscence or destruction caused by SWCs was clearly observed. **B**, Incidence of SWCs. Numbers of diabetic rats with SWCs (filled bar) were shown ($n = 8$ for each group). The open bar shows rats without SWCs. The incidence of SWCs was significantly lower in the EP4 group. $*P < .01$ vs control.

Study 2: Prevention of SWCs by the EP4 Agonist

Assessment of sternal bone regeneration

INCIDENCE OF SWCs. SWCs were diagnosed by using a soft x-ray film of the sternum (Figure 2, A). Destruction or dehiscence of the separated original sternum was clearly observed in the rats with SWCs. Four weeks after the operation, SWCs developed in 5 rats in the control group

but in only 1 rat in the EP4 group (63% and 13%, $P < .01$; Figure 2, B). All the rats with SWCs had macroscopic abscesses in the anterior mediastinum.

STERNAL HEALING AFTER STERNOTOMY. Regarding the sternal healing without the SWCs in each group, dehiscence of the separated original sternum was observed in the control group (Figure 3, B). In contrast, sternal union and almost complete bone healing was observed in the EP4 group (Figure 3, A), and thus almost complete bone healing was observed only in the EP4 group.

DUAL-ENERGY X-RAY ABSORPTIOMETRY. As illustrated in Figure 4, A, the BMC of the regenerated sternum assessed on the basis of DEXA was higher in the EP4 group than in the control group (71.7 ± 12.1 vs 48.9 ± 11.7 mg, $P < .01$). The BMD of the regenerated sternum was also higher in the EP4 group than in the control group (66.8 ± 14.6 vs 47.9 ± 6.3 mg/mm², $P < .05$).

HISTOLOGIC ANALYSIS. Figure 5 shows histologic sections of the sternum 4 weeks after the operation. The rats in the EP4 group showed almost completely healed sternums filled with regenerated bone tissue and bone marrow (Figure 5, A), except 1 rat that showed poor sternal healing because of SWCs. Conversely, slight bone regeneration between the incised original sternum was observed in the control group (Figure 5, B).

Assessment of peristernal blood perfusion

LASER DOPPLER PERFUSION ANALYZER. Figure 6, A, shows the peristernal blood perfusion immediately after the removal of the BITAs and 4 weeks after the operation in each group. The blood perfusion immediately after BITA removal previously showed a similar decrease in both groups

Figure 3. Effects of the EP4 agonist on sternal regeneration as shown by means of soft x-ray films. Soft x-ray films show sternal regeneration 4 weeks after the operation. **A**, Sternum was treated with the EP4 agonist. **B**, No treatment (control). Sternal dehiscence was observed only in the control group (white arrows), whereas sternal dehiscence clearly disappeared in the EP4 group.

Figure 4. Quantitative and qualitative analysis of sternal bone regeneration assessed by means of dual-energy x-ray absorptiometry. Bone mineral content (BMC; A) and bone mineral density (BMD; B) 4 weeks after the operation are shown. Both the BMC and BMD significantly increased only in the EP4 group. * $P < .01$ and # $P < .05$ vs control.

($50.6\% \pm 8.3\%$ and $51.3\% \pm 6.5\%$ of the preoperative level, respectively). Four weeks after the operation, there was no significant difference observed in the peristernal blood perfusion between groups ($77.8\% \pm 9.1\%$ vs $73.9\% \pm 10.3\%$). However, the blood perfusion had naturally recovered in both groups when compared with that immediately after BITA removal.

HISTOLOGIC ANALYSIS. Figure 6, B, shows the number of arterioles and capillaries per unit area around the sternum 4 weeks after the operation in both groups. No difference was observed in the number of vessels between the groups (17.2 ± 3.8 vs 15.5 ± 4.5 vessels per unit area).

Discussion

The present study demonstrates the utility of the PGE₂ EP4 receptor-selective agonist for the acceleration of sternal

Figure 6. Evaluation of peristernal blood perfusion recovery. A, Laser Doppler perfusion image analyzer measurements immediately after removal of BITA and 4 weeks after the operation are shown. The EP4 agonist did not recover the blood perfusion in the peristernum in both groups. B, The EP4 agonist did not increase the peristernal number of vessels in both groups. BITA, Bilateral internal thoracic artery.

healing and reduction in the incidence of SWCs after median sternotomy with the removal of BITAs in diabetic rats. Soft x-ray film showed complete sternal healing only in the rats treated with the EP4 agonist. The agonist significantly increased both the BMC and BMD of the sternum, although it did not increase the peristernal blood perfusion. An important observation was that the EP4 agonist decreased the incidence of SWCs. This therapeutic approach would potentially not only facilitate the wider application of BITAs in coronary artery bypass surgery, particularly in high-risk diabetic patients, but also enable fast-track recovery.

Slow release of the PGE₂ EP4 receptor-selective agonist from the PLGA microspheres might be a more effective and less invasive strategy for bone regeneration than the sys-

Figure 5. Histologic cross-sections of hematoxylin and eosin-stained sternum were obtained 4 weeks after the operation. A, Regenerated sternum with new bone was observed in the EP4 group. B, Regenerated sternum with new bone was observed partially in the control group. NB, New bone with bone marrow. (Original magnification $1\times$.)

temic administration of PGE₂. A noteworthy finding is that the new bone induced by PGE₂ was mainly composed of fibrous tissues, whereas the trabeculae of the new bone induced by the EP4 agonist were well connected and sufficiently calcified and yielded substantial strength to the new bone.¹⁵ In addition, the systemic use of PGE₂ is limited by undesirable effects, such as hypotension, diarrhea, contraction of the uterus, or thickening of the intestinal epithelium. Because the EP4 agonist acts selectively on one subtype of the EP4 receptors, its use is expected to reduce several adverse actions that are caused because of the systemic administration of PGE₂. Furthermore, its slow-release system from the PLGA would enable dose reduction of the agonist and might additionally contribute in the avoidance of side effects. We expect the topical and sustained use of the EP4 agonist to result in satisfactory bone regeneration without systemic complications in human subjects.

Previously we have reported the utility of bFGF for the prevention of SWCs after median sternotomy with BITA removal in rats.^{9,10} However, the EP4 agonist might be more effective in bone regeneration than bFGF. Callus remodeling is accelerated by bFGF by means of both osteoblastic callus formation and osteoclastic callus resorption, thereby resulting in bone regeneration.^{18,19} On the other hand, the EP4 agonist plays a greater role in osteogenic processes than bFGF.¹⁵ The EP4 agonist induced callus formation and increased the volume of both cancellous bone and osteoidas, as well as the number of osteoblasts. In the previous series, only the BMC was increased by bFGF⁹; however, in the present study the EP4 agonist increased both the BMC and BMD, thereby indicating the superiority of the EP4 agonist as an osteogenic agent when compared with bFGF.

SWCs could not be completely prevented with bFGF alone⁹ or with the EP4 agonist alone; however, a combination of bFGF and the EP4 agonist might support each other. Although bFGF significantly recovered the peristernal blood perfusion, it increased only the BMC in the ischemic sternum of diabetic rats, whereas the EP4 agonist increased both the BMC and BMD without recovery of the peristernal blood perfusion. Therefore a combination of the angiogenic effect of bFGF and the osteogenic effect of the EP4 agonist might provide more satisfactory results.

We used streptozotocin-induced diabetic rats in the present study. This diabetic model showed delayed wound healing, which might have been caused by a small vascular change and impaired leukocyte physiology caused by hyperglycemia. Hyperglycemia followed by vascular alterations is the most common cause of morbidity and mortality in diabetic patients. Several studies have reported on the endothelial dysfunction and functional alterations in the microcirculation of diabetic patients, such as increased vascular permeability, alterations in erythrocyte velocity, se-

questration (entrapment) of leukocytes in the microcirculation, and morphologic alterations, such as altered vascular density.²⁰ Leukocytes that are adherent to vascular endothelium have been shown to cause capillary occlusion and endothelial cell apoptosis.²¹ In addition, hyperglycemia-induced neutrophil dysfunction is strongly correlated with increased susceptibility to infection and delayed wound healing.²²

Recent clinical studies have reported that harvesting of the BITAs in a skeletonized fashion lowers the risk of sternal infection, even in patients with diabetes.^{7,8} Harvesting the ITA in a skeletonized fashion might preserve greater collateral blood flow than that in a pedicled fashion.^{23,24} However, all collateral vessels cannot be preserved; this is due to anatomic and not technical reasons.^{25,26} Three types of collateral vessels were identified to possess the potential to supply blood to the sternum after the mobilization of the internal thoracic artery. The blood flow to the sternum was preserved after the ligation of the collateral vessels, such as the sternal-intercostal branch, whereas the ligation of the collateral vessels, such as the sternal-perforating branch, or noncollateral vessels resulted in loss of the blood supply.²⁵ Therefore, even careful skeletonization destroys the collaterals from the lateral chest wall to the sternum. This trial with the EP4 agonist is a novel regenerative approach for the devascularized sternum, irrespective of the harvesting techniques used by surgeons.

We used fibrin glue as a scaffold for the PLGA microspheres that were incorporated with the EP4 agonist. Fibrin glue, a composite of fibrinogen and thrombin, is a suitable biologic vehicle for cell transplantation because it has been proved to be biocompatible and biodegradable and is capable of binding to cells.^{27,28} It is also used as a slow and local delivery vehicle for drugs such as losartan.²⁸ PLGA, a delivery vehicle for the EP4 agonist, itself is commonly used as a slow-release carrier for drugs. First, we dispersed the microspheres containing the EP4 agonist in a surfactant (Tween 80) and injected it directly into the anterior mediastinal space; however, the osteogenic effect was weak (data not shown). This might be due to the rapid spread of the microspheres in the thoracic cavity. Then we applied the fibrin glue as a scaffold for the PLGA microspheres and obtained satisfactory results. The fibrin glue itself did not exhibit osteogenic effects in this devascularized sternum model of diabetic rats.

The present study has several limitations. First, the model used in this study has a narrow and thin sternum, and its anatomic features differ from those of human subjects. The structural differences might influence sternal healing induced by the EP4 agonist. Additional investigations with large-animal models are required before studies on human subjects. Second, we did not examine the most suitable

release periods for sternal healing by changing the release profile.

In conclusion, the PGE₂ EP4 receptor-selective agonist accelerated healing of the devascularized sternum and decreased the incidence of SWCs, even in diabetic rat models. This method might potentially enable the wider application of BITAs in coronary artery bypass surgery in high-risk patients with diabetes and provides excellent long-term results. Furthermore, fast healing of the sternum shortens the patients' hospital stay, considerably decreases health care costs, and facilitates the patients' return to work or social activities.

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References

1. Stahle E, Tammelin A, Bergstrom R, Hambreus A, Nystrom SO, Hansson HE. Sternal wound complications—incidence, microbiology and risk factors. *Eur J Cardiothorac Surg*. 1997;11:1146-53.
2. Sharma R, Puri D, Panigrahi BP, Viridi IS. A modified parasternal wire technique for prevention and treatment of sternal dehiscence. *Ann Thorac Surg*. 2004;77:210-3.
3. Sarr MG, Gott VL, Townsend TR. Mediastinal infection after sternotomy. *Ann Thorac Surg*. 1984;38:415-23.
4. Grossi EA, Culliford AT, Krieger KH, Kloth D, Press R, Baumann, et al. A survey of 77 major infectious complications of median sternotomy: a review of 7949 consecutive operative procedures. *Ann Thorac Surg*. 1985;40:214-21.
5. Zacharias A, Habib RH. Factors predisposing to median sternotomy complications. *Chest*. 1996;110:1173-8.
6. Crabtree TD, Codd JE, Fraser VJ, Bailey MS, Olsen MA, Damiano RJ Jr. Multivariate analysis of risk factors for deep and superficial sternal infection after coronary artery bypass grafting at a tertiary care medical center. *Semin Thorac Cardiovasc Surg*. 2004;16:53-61.
7. Peterson MD, Borger MA, Rao V, Peniston CM, Feindel CM. Skeltonization of bilateral internal thoracic artery grafts lowers the risk of sternal infection in patients with diabetes. *J Thorac Cardiovasc Surg*. 2003;126:1314-9.
8. Lev-Ran O, Mohr R, Amir K, Matsa M, Nehser N, Locker C, et al. Bilateral internal thoracic artery grafting in insulin-treated diabetics: should it be avoided? *Ann Thorac Surg*. 2003;75:1872-7.
9. Iwakura A, Tabata Y, Miyao M, Ozeki M, Tamura N, Ikai A, et al. Novel method to enhance sternal healing after harvesting bilateral internal thoracic arteries with use of basic fibroblast growth factor. *Circulation*. 2000;102(suppl III):III307-11.
10. Iwakura A, Tabata Y, Tamura N, Doi K, Nishimura K, Nakamura T, et al. Gelatin sheet incorporating basic fibroblast growth factor enhances healing of devascularized sternum in diabetic rats. *Circulation*. 2001;104(suppl I):I325-9.
11. Narumiya S, Sugimoto Y, Ushikubi F. Prostanoid receptors: structures, properties, and functions. *Physiol Rev*. 1999;79:1193-226.
12. Kawaguchi H, Pilbeam CC, Harrison JR, Raisz LG. The role of prostaglandins in the regulation of bone metabolism. *Clin Orthop*. 1995;313:36-46.
13. Weinreb M, Machwate M, Shir N, Abramovitz M, Rodan GA, Harada S. Expression of the prostaglandin E₂ (PGE₂) receptor subtype EP₄ and its regulation by PGE₂ in osteoblastic cell lines and adult rat bone tissue. *Bone*. 2001;28:275-81.
14. Weinreb M, Grosskopf A, Shir N. The anabolic effect of PGE₂ in rat bone marrow cultures is mediated via the EP4 receptor subtype. *Am J Physiol*. 1999;276:376-83.
15. Yoshida K, Oida H, Kobayashi T, Maruyama T, Tanaka M, Katayama T, et al. Stimulation of bone formation and prevention of bone loss by prostaglandin E EP4 receptor activation. *Proc Natl Acad Sci U S A*. 2002;99:4580-5.
16. Taniyama Y, Morishita R, Aoki M, Nakagami H, Yamamoto K, Yamazaki K, et al. Therapeutic angiogenesis induced by human hepatocyte growth factor gene in rat and rabbit hind limb ischemia models: preclinical study for treatment of peripheral arterial disease. *Gene Ther*. 2001;8:181-9.
17. Riyamongkol P, Zhao W, Liu Y, Belayev L, Busto R, Ginsberg MD. Automated registration of laser Doppler perfusion images by an adaptive correlation approach: application to focal cerebral ischemia in the rat. *J Neurosci Methods*. 2002;122:79-90.
18. Nakamura T, Hara Y, Tagawa M, Tamura M, Yuge T, Fukuda H, et al. Recombinant human basic fibroblast growth factor accelerates fracture healing by enhancing callus remodeling in experimental dog tibial fracture. *J Bone Miner Res*. 1998;13:942-9.
19. Rifkin DB, Moscatelli D. Recent developments in the cell biology of basic fibroblast growth factor. *J Cell Biol*. 1989;109:1-6.
20. Algenstaedt P, Schaefer C, Biermann T, Hamann A, Schwarzloh B, Greten H, et al. Microvascular alterations in diabetic mice correlate with level of hyperglycemia. *Diabetes*. 2003;52:542-9.
21. Miyahara S, Kiryu J, Yamashiro K, Miyamoto K, Hirose F, Tamura H, et al. Simvastatin inhibits leukocyte accumulation and vascular permeability in the retinas of rats with streptozotocin-induced diabetes. *Am J Pathol*. 2004;164:1697-706.
22. Kannan Y, Tokunaga M, Moriyama M, Kinoshita H, Nakamura Y. Beneficial effects of troglitazone on neutrophil dysfunction in multiple low-dose streptozotocin-induced diabetic mice. *Clin Exp Immunol*. 2004;137:263-71.
23. Cohen AJ, Lockman J, Lorberboym M, Bder O, Cohen N, Medalion B, et al. Assessment of sternal vascularity with single photon emission computed tomography after harvesting of the internal thoracic artery. *J Thorac Cardiovasc Surg*. 1999;118:496-502.
24. Athanasiou T, Crossman MC, Asimakopoulos G, Cherian A, Weerasinghe A, Glenville B, et al. Should the internal thoracic artery be skeletonized? *Ann Thorac Surg*. 2004;77:2238-46.
25. Jesus RA, Acland RD. Anatomic study of the collateral blood supply of the sternum. *Ann Thorac Surg*. 1995;59:163-8.
26. Gupta M, Sodhi L, Sahni D. Variations in collateral contributions to the blood supply to the sternum. *Surg Radiol Anat*. 2002;24:265-70.
27. Keller J, Andreassen TT, Joyce F, Knudsen VE, Jorgensen PH, Lucht U. Fixation of osteochondral fractures. Fibrin sealant tested in dogs. *Acta Orthop Scand*. 1985;56:323-6.
28. Moon MC, Molnar K, Yau L, Zahradka P. Perivascular delivery of losartan with surgical fibrin glue prevents neointimal hyperplasia after arterial injury. *J Vasc Surg*. 2004;40:130-7.