

Acta Medica Okayama

Volume 47, Issue 2

1993

Article 9

APRIL 1993

Beneficial effect of EPC-K1 on the survival of warm ischemic damaged graft in rat cardiac transplantation.

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Beneficial effect of EPC-K1 on the survival of warm ischemic damaged graft in rat cardiac transplantation.*

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Abstract

A newly introduced compound, EPC-K1, represents a phosphate diester linkage of vitamin E and vitamin C. The effect of EPC-K1 on the reperfusion injury was evaluated in a heterotopic cardiac transplantation model using syngenic combination rats. Prior to the warm ischemia, 12mg EPC-K1/kg was administered intravenously to donor rats. After 15 min of warm ischemic time, hearts were harvested and perfused with 4 degrees C saline. After completion of the transplantation, recipient rats were also treated with intravenous 12 mg EPC-K1/kg, before reperfusion. Saline was used instead of EPC-K1 for both donors and recipients in the control group. On the 7th post-transplantation day, graft survival was 7 out of 8 in EPC-K1 group, versus 1 out of 9 in the control group ($p < 0.001$). Thiobarbituric acid-reactive substance levels in the recipient serum, three hours after reperfusion, were significantly limited, in the group in which EPC-K1 was administered only to donors. But it was not possible to clarify whether the effect of EPC-K1 is primarily at the donor or recipient levels at this time. These results indicate that EPC-K1 may reduce reperfusion injury after cardiac transplantation. This beneficial effect may be mediated by the hydroxyl radical scavenging properties of EPC-K1.

KEYWORDS: EPC-K, cardiac transplantation, free redical scavenger, reperfusion injury, thiobarbituric acidreactive substances

*PMID: 8506749 [PubMed - indexed for MEDLINE]

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300 g (purchased from Seiwa Exp. Animal Inc., Fukuoka, Japan) were used as donors and recipients. The total number of rats used was 79.

Cardiac grafting. Intra-abdominal heterotopic cardiac transplantation was performed using the Ono-Lindsey method (6). Donor rats were first anesthetized by inhalation of ether. The donor ascending aorta was transected. After 15 min of warm ischemic time, the graft was perfused with 4 °C saline through the transected aorta. The recipient rats were anesthetized by ether inhalation and laparotomized. The intra-abdominal cardiac transplantation was completed with aorto-aortic end-to-side anastomosis and pulmonary arterio-caval end-to-side anastomosis.

Design of experiment 1 (Fig. 2). In the EPC-K₁ group, 12 mg EPC-K₁/kg was administered intravenously to the donor rats, 5 min before the transection of the donor aorta. After completion of the transplantation, recipient rats were also treated with intravenous EPC-K₁, 12 mg EPC-K₁/kg, 5 min before reperfusion. In the control group, saline was used instead of EPC-K₁,

for both donors and recipients. After transplantation, rats were kept in individual cages and received standard rat chow and water *ad libitum*.

Evaluation of graft function. Graft survival was assessed by means of graft pulsation and ECG. Graft viability was assessed at three levels of B0, B1 and B2. B0 represents neither graft pulsation nor graft electrical activity. In B1, weak graft pulsation can be palpated, but no graft electrical activity is detected by recipient ECG as shown in Fig. 3A. B2 represents the strong pulsation. In B2, two different QRS patterns caused by the recipient's heart and the graft are noted on recipient ECG (Fig. 3B). On the 7th day post-transplantation, recipients were sacrificed and grafts were harvested for pathological examination.

Design of experiment 2. Experiment 2 consists of the measurement of thiobarbituric acid-reactive substance (TBARS) levels (7) of recipient serum and graft myocardium, 3 h after reperfusion of transplanted heart. Five groups made up this experiment. In the S + S group, 1 ml saline was administered to

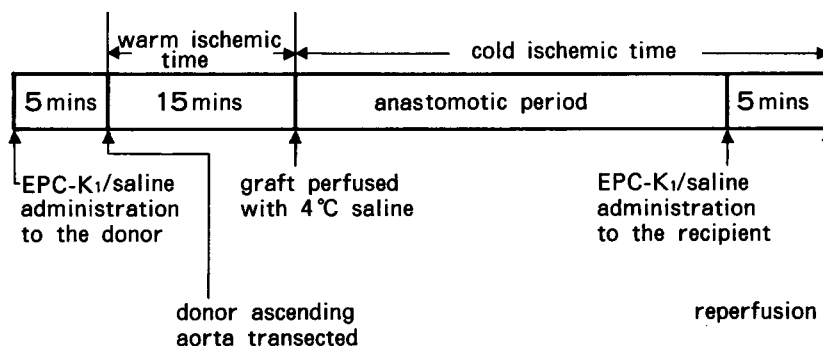


Fig. 2 Experiment protocol (experiment 1).

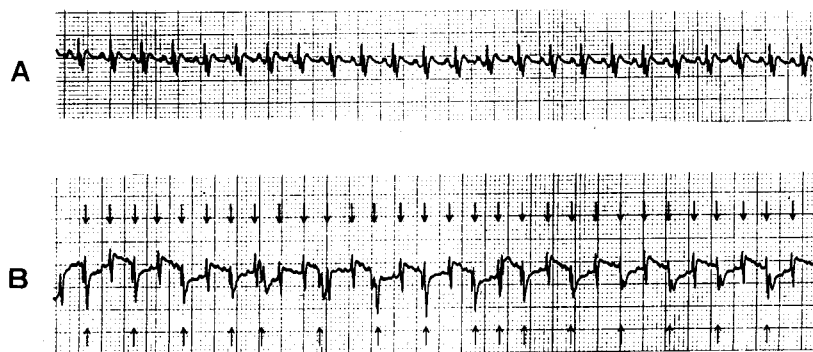


Fig. 3 ECG patterns of recipients with grafts of viability degrees of B1 (A) and B2 (B). Graft viability degrees are described in the text.

donors and recipients. In the E + E group, EPC-K₁ was administered to both donors and recipients. In the S + E group, 1 ml saline was administered to donors and EPC-K₁ to recipients. In the E + S group, EPC-K₁ to donors and 1 ml saline to recipients. Nontreated male Wistar rats served as controls.

Statistics. Statistical analysis was performed using Student's *t*-test, and differences were considered significant when $p < 0.05$.

Results

Isograft survival on the seventh post-transplantation day. There were no significant differences in the mean cold ischemic time, which included the anastomotic period, between the EPC-K₁ group (52.6 ± 2.82 min., $n = 8$) and the control (53.9 ± 5.46 min., $n = 9$). The time course of graft viability is shown in Fig. 4. The presence of strong contractions (B2) on the 7th post-transplantation day occurred in 7 out of 8 grafts in the EPC-K₁ group, but in only 1 out of 9 in the control group. The difference was statistically significant ($p < 0.001$).

Microscopic findings of 2 transplanted hearts. The

microscope used was Fluophoto Nikon. Microscopic findings of a B2 graft from the EPC-K₁ group are shown in Fig. 5. Graft myocardium was well preserved, and no bleeding was noted in the myocardium. Microscopic findings of the B2 graft from the control group are shown in Fig. 6. Graft myocardium became thin, and marked bleeding and fibrosis were noted in the myocardium. There also was a large thrombus in the left ventricle.

TBARS levels three hours after grafting. TBARS levels in recipient serum and graft myocardium are shown in Figs. 7-8. In the E + S group, the mean TBARS level of recipient serum was significantly lower than in the S + S group. In the E + E and S + E groups, however, the limited deviations of the mean TBARS levels of recipient serum were not significant. The spread of the mean TBARS levels of graft myocardium was not significant in the 5 groups.

Discussion

Ischemia followed by reperfusion causes some injury to the reperfused myocardium and this phenomenon is

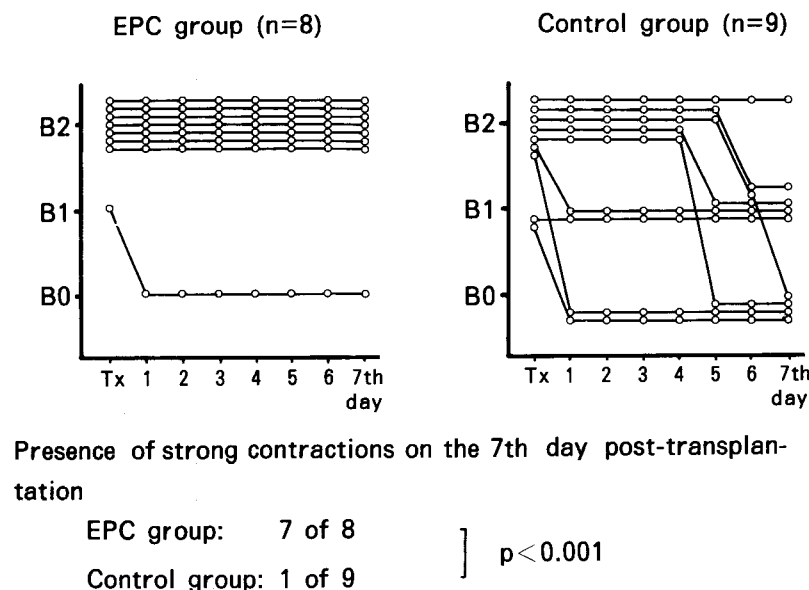


Fig. 4 Graft contraction quality after transplantation.



Fig. 5 Microscopic view of a harvested B2 graft from the EPC-K₁ group. (Azan stain $\times 10$)

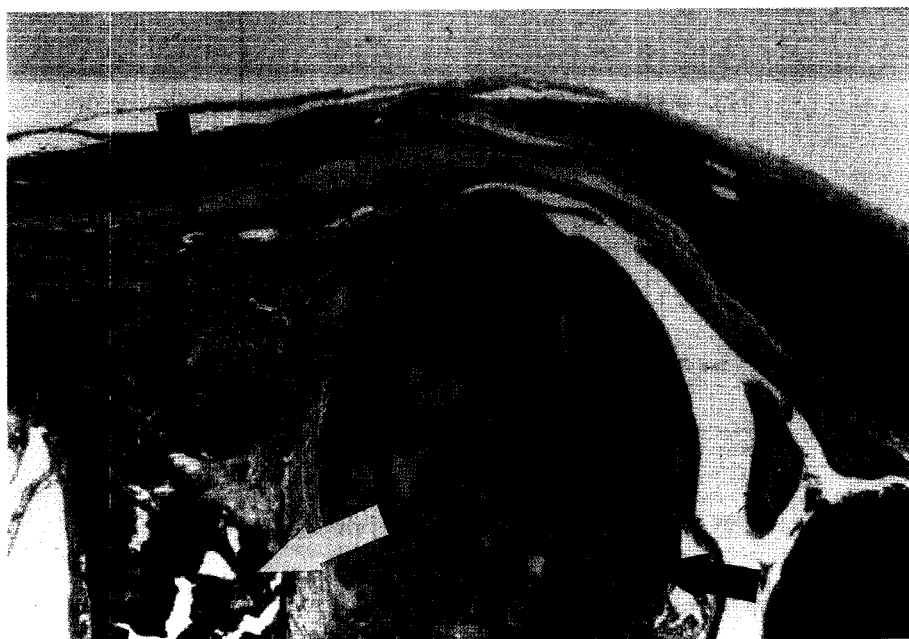


Fig. 6 Microscopic view of the harvested B2 graft from the control group. (Azan stain $\times 10$) Marked bleeding (green arrow) and fibrosis (orange arrow) were noted in the myocardium. There also was a large thrombus (black arrow) in the left ventricle.

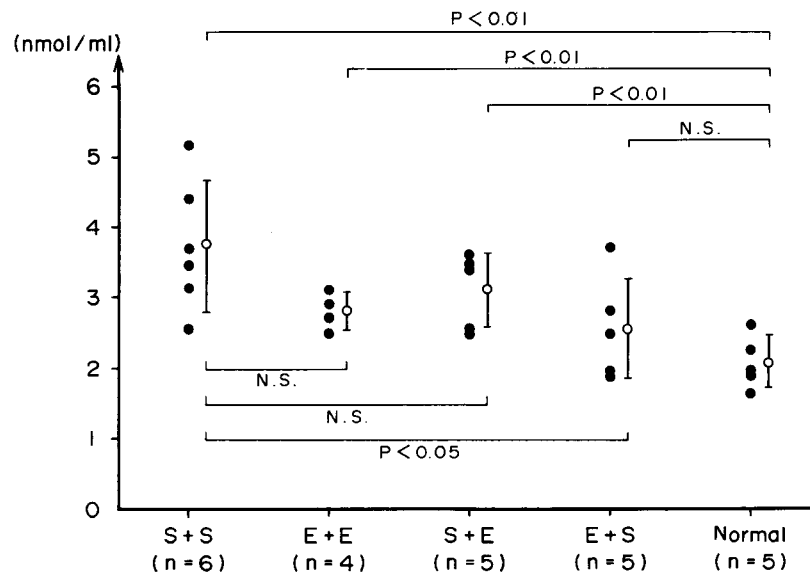
Effect of EPC-K₁ on Cardiac Transplantation

Fig. 7 Recipient serum thiobarbituric acid-reactive substance (TBARS) levels, 3h after reperfusion.
 S + S ; 1 ml saline to both donors and recipients.
 E + E ; 12mg EPC-K₁/kg to both donors and recipients.
 S + E ; 1 ml saline to donors and 12mg EPC-K₁/kg to recipients.
 E + S ; 12mg EPC-K₁/kg to donors and 1 ml saline to recipients.
 Normal; non-treated male Wistar rats as control.

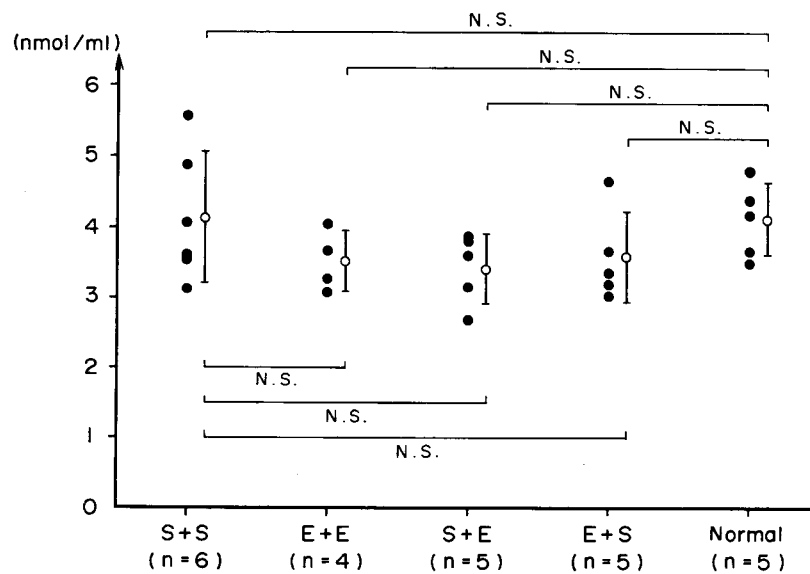


Fig. 8 Graft myocardial TBARS levels, 3h after reperfusion. Fashion of treatment is the same as in Fig. 7. TBARS: See Fig. 7.

termed reperfusion injury (8). The mechanistic determinants of reperfusion injury are multifactorial and are not

yet completely understood (9, 10). The multifactorial determinants include calcium overload (11), mitochondrial

dysfunction (12), arachidonic acid metabolites (13), and defective myocardial lipid metabolism (14). It has been reported that oxygen free radicals contribute to myocardial injury associated with myocardial ischemia and reperfusion (1-3). Oxygen free radical-induced cytotoxicity depends largely on the subsequent production of a highly reactive species of oxygen free radical, the hydroxyl radical which has been reported to be one of the most toxic compounds (3, 15). There are no known enzyme systems that can scavenge excessive quantities of hydroxyl radical (16).

Vitamin E, α -tocopherol, is a lipid soluble vitamin. It has been recently documented that vitamin E could have both protective and therapeutic roles in reperfusion injury. Heart tissue is richer in vitamin E than tissue in the liver and approximately three-fold greater than that in noncardiac striated muscle (17, 18). In contrast, the superoxide dismutase level in the human heart is about 10 % of that in the liver, and cardiac catalase is present at approximately 2 % of the hepatic level (19). Vitamin E is apparently the only significant peroxy-radical trapping, chain-breaking antioxidant in the heart. Vitamin E demonstrates cardioprotective effects in some animal models of myocardial ischemia-reperfusion when administered prior to the ischemic period (20, 21). It is thought that this property is expressed in the stabilization of cardiac membranes against oxidative stress (22). Vitamin C also has the antioxidant and prooxidant properties (23). Many studies on the interaction of vitamin E and vitamin C have been presented (24-28). According to those studies, administration of an optimal combination of vitamin E and vitamin C may substantially enhance tissue protection.

EPC-K₁ is a newly introduced compound and represents the phosphate diester linkage of vitamin E and vitamin C. EPC-K₁ has a hydroxyl radical scavenging effect, and the effect is dose dependent (4). EPC-K₁ also has anti-inflammatory properties through its phospholipase A₂ inhibitory effect (5). Moreover, EPC-K₁ is amphipathic, that is, it is soluble in both water and lipid; it, therefore, may be used intravenously and it is rapidly absorbed in many organ tissues.

In experiment 1, the effect of EPC-K₁ was evaluated in the prevention of reperfusion myocardial injury through a heterotopic cardiac transplantation model using the heart grafts exposed to warm ischemia. Our experiment revealed that EPC-K₁ significantly improved graft survival. This beneficial effect might be partially attributed to the hydroxyl radical scavenging properties of EPC-K₁.

Experiment 2 was designed to clarify whether the effect of EPC-K₁ is primarily at the donor or recipient levels. In the E + S group, the mean TBARS level of recipient serum was significantly limited compared with that in S + S group. But, that in the E + E group had no significant limitation probably due to small numbers. Moreover, there were no significant differences in the spread of the mean TBARS levels of graft myocardia. Therefore, it was not possible to identify which levels EPC-K₁ mainly affect.

In conclusion, EPC-K₁ appears to reduce reperfusion myocardial injury after cardiac transplantation in the rat model. Further studies are required to more precisely define the mechanism of apparent protection and to ascertain the suitability of EPC-K₁ for use in humans.

Acknowledgments. The authors are grateful to Dr. K. Ogata, Senju Pharmaceutical Co., Ltd., Osaka, for providing solution of EPC-K₁.

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Received October 13, 1992; accepted November 25, 1992.