Apelin-13 Attenuates Ischemic Changes in A Rat Model of Myocardial Infarction via Increasing Circulating Angiogenic Factors

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Abstract

Ischemic heart disease is a global health concern in the present day with limited therapies. Extensive efforts have been devoted to find therapies that promote angiogenesis and enhance perfusion to the ischemic myocardium. The aim of the present study was to look into the effects of apelin-13 on circulating angiogenic factors after myocardial infarction in rats. Forty adult male rats were randomly assigned into four equal groups (n=10); Sham operated, Sham operated + Apelin 13, myocardial infarction (MI) group and MI + Apelin 13. Infarction size, serum level of cardiac enzymes and serum levels of angiogenic factors; insulin like growth factor one (IGF-1), transforming growth factor-beta (TGF-β) and vascular endothelial growth factor (VEGF) were assessed 28 days after apelin administration. Compared to sham operated group, induction of myocardial infarction resulted in significant increase in infarction size from zero to 45.85 $\pm$ 2.94, cardiac biomarkers (CK-MB and LDH) from 3.19 $\pm$ 0.62 ng/ml and 24.87 $\pm$ 3.48 IU/L to 27.75 $\pm$ 1.24 ng/ml and 98.82 $\pm$ 3.24 IU/L respectively (P < 0.05) as well as serum angiogenic factors (IGF-1, VEGF and TGF-β levels) as they were increased from 3.44 $\pm$ 0.46 ng/ml, 6.33 $\pm$ 0.88 ng/ml and 145.09 $\pm$ 4.80 pg/ml to 9.86 $\pm$ 0.92, 12.67 $\pm$ 0.77 and 338.77 $\pm$ 9.56 respectively. Apelin-13 treatment induced a significant increase in serum angiogenic factors and reduced the ischemic changes in the myocardium when compared to the MI group. These results indicated that apelin treatment protects against myocardial infarction via increased circulating angiogenic growth factors therefore, apelin may be a new therapeutic target in myocardial infarction.

Keywords: Apelin-13, Myocardial infarction, Angiogenesis

1. Introduction

Myocardial infarction is a fundamental public health problem and the leading cause of mortality in both developed and developing countries. According to the world health organization (WHO) report, heart disease and stroke will become the leading causes of death and disability worldwide by the year 2025 [1]. Hence, in light of the above-alarming statistics, researches on producing potent therapeutics for use in heart diseases are getting more focused [2].

Myocardial infarction (MI) is an acute condition that occurs by occlusion of coronary artery, resulting in imbalance between coronary blood supply and myocardial demand, cardiac muscle damage and loss of cardiomyocytes [3]. Left anterior descending artery is the main blood-supply vessels for the left ventricle anterior wall, left ventricular lateral wall and interventricular septum. Coronary artery ligation of the left anterior descending coronary artery is the most commonly used method in the study of myocardial infarction and myocardial ischemia pharmacodynamics [4].

An interesting hypothesis is that the development of new blood vessels from preexisting vascular bed (angiogenesis) or the remodeling of preexisting collaterals has the potential to salvage the ischemic myocardium and improve the outcome after AMI [5]. Angiogenic growth factors exert a major role in blood vessel formation by aiding cell proliferation, migration and adhesion. These growth factors are produced by various cell types and include a wide range of proteins like vascular endothelial growth factor (VEGF), transforming growth factor (TGF), fibroblast growth factor (FGF), platelet derived growth factor (PDGF), insulin like growth factor-1 (IGF-1), angiopoietins (Ang) and others [6]. A number of angiogenic growth factors are known to be expressed during MI [7].

Although a number of drugs have been widely used to treat myocardial ischemia, the usefulness of these drugs has been limited due to their serious adverse effects, such as cardiac depression or pro-arrhythmic effects thus there is great interest in the development of new types of cardioprotective drugs, which may attenuate the effects of myocardial infarction in clinical practice [8].

Apelin, a novel bioactive peptide, was initially identified as an endogenous ligand for the orphan G protein-coupled, sharing similarities with the angiotensin II type 1 receptor pathway [9]. It was originally isolated from bovine stomach tissue extracts in 1998 by *Tatemoto et al.* [10]. Several active fragments of apelin have been identified (apelin-36, apelin-19, apelin-17, apelin-16, apelin-13, and apelin-12). Apelin-13 and apelin-36 are considered the most active isoforms with the greatest activity on the cardiovascular homeostasis [11].

Apelin is expressed in various tissues like hearts, brain, lungs, kidneys, liver, gastrointestinal tract, adipose tissue, adrenal glands, endothelium and human plasma. It mediates a wide range of physiological and pathological functions including dilatation of arteries, systolic effect, powerful inotrope, regulation of fluid homeostasis, and cell proliferation [12]. Recent experiments in animal models indicate that the cardiovascular system is the main target of the apelin - apelin receptor system [13].

We aimed in this study to find out the cardioprotective effect of apelin in a rat model of post MI and its effect on angiogenesis.

2. Materials and Method

2.1 Animals

Forty male albino Wistar rats of 8-12 weeks of age and weighing between 200-250g were purchased from the animal house of Faculty of Medicine, Cairo University, Egypt. All procedures were approved by the Institutional Ethical Committee for Animal Care and Use of the Faculty of Medicine, Benha University. The animals were acclimatized over a week to standard laboratory conditions (22-24° C surrounding temperature, 40-60% relative humidity and 12-12 h light-dark cycle).

2.2 Experimental design

The animals were randomly divided into four equal groups (n = 10): (a) Sham operated group (b) sham operated group + apelin-13 (c) MI group and (d) MI + apelin-13 group. MI was induced in rats by ligation of the left anterior descending coronary artery (LAD) as described earlier by Pfeffer et al. A left thoracotomy was performed via the 4th

intercostal space, and the lungs were retracted to expose the heart. After the pericardium was opened, the LAD artery was ligated, 2 mm from its origin using a 4-0 silk (*Ethicon, Johnson and Johnson, U.S.A.*). Ligation was considered successful when the anterior wall of the LV turned pale. The chest was closed with a 2-0 silk (*Ethicon, Johnson and Johnson, U.S.A.*) after it was gently pressed to expel air from the cavity to avoid a pneumothorax. Once awake, the rats were injected intramuscularly with buprenorphine HCl (0.01 mg/kg body weight) to reduce the pain during recovery. The sham operated group underwent a similar procedure except that the suture was not tightened around the LAD artery [14]. Apelin-13 (1.5 μ g/kg/day) or saline (*Sigma, St Louis, MO, USA*) were intraperitoneally (i.p.) injected after the onset of myocardial infarction once daily for 28 days [15].

By the end of the experimental period, all rats were the rats were anesthetized by with xylazine (10 mg/kg body weight) and Ketamine (100 mg/kg body weight) (*Sigma, St Louis, MO, USA*). Blood sample was drawn from the abdominal aorta and was allowed to clot at room temperature and sera were separated by centrifugation at 3000 rpm for 15 min and stored at -80˚C for biochemical assessment of cardiac biomarkers and circulating angiogenic factors and the hearts were excised for assessment of the infarction size.

2.3 Assessment of the infarction size

The excised beating heart was submerged in cold KCl to achieve diastolic arrest. The right ventricle and both atria were excised to isolate the left ventricle. The left ventricle is sectioned into thin transverse slices and submerged in a 1.5% triphenyl tetrazolium chloride (TTC) stain in phosphate buffer, PH 7.4, for 15 minutes at 37° C. TTC stain form red color in the presence of intact dehydrogenase enzyme system thus the infarction areas were not stained red. TTC stained and non-stained areas were traced on clear plastic sheets fixed on an E.C.G paper to be counted in mm2. The infraction size % was calculated as the total of infract area divided by the total of surface areas of all the slices [16].

2.4 Determination of cardiac biomarkers

Serum lactate dehydrogenase (LDH) was determined enzymatically with pyruvate as substrate by using standard kits from (*BioSystems S.A., Barcelona, Spain*). Serum creatine kinase-MB (CK-MB) activity was assessed by an immune-inhibition method using standard kits from (*BioSystems S.A., Barcelona, Spain*) through the rate of NADPH formation by means of the hexokinase and glucose-6-phosphate dehydrogenase coupled reactions [17].

2.5 Insulin-like growth factor-1 (IGF-1), transforming growth factor- β (TGF- β) and vascular endothelial growth factor (VEGF) assay

Commercially available enzyme-linked immunosorbant assay (ELISA) kit (*R&D Systems, Inc., Minneapolis, MN, USA*) was used in this assay. The micro titre plate provided in this kit was pre-coated with a primary antibody specific to each factor. Standards or samples are then added to the appropriate micro titre plate wells together with a biotin-conjugated specific secondary polyclonal antibody. Avidin conjugated to horseradish peroxidase and a 3,3'5,5' tetramethyl-benzidine substrate completed the immuno-reaction sequence that was ended with the addition of a sulphuric acid solution. Color change was measured at 450 nm [17].

2.6 Statistical analysis

All analyses were performed using the program Statistical Package for Social Sciences version 19 (*SPSS Inc, Chicago, IL, USA*). The data were presented as the mean (M) ± standard deviation (SD). Comparisons between groups were analyzed by using one-way Analysis of Variance (ANOVA) followed by post-hoc LSD test. Probability of chance (P value) < 0.05 was considered statistically significant.

3. Results

3.1 Effect of Apelin-13 on the infarction size and cardiac biomarkers (CK-MB and LDH) (Table 1 and fig. 1 & 2)

The survival rates at the end of the observation of the rats were 100% (10/10) in the sham groups, 80% (8/10) in MI group and 90% (9/10) in the MI + apelin group respectively. Table 1 and figure 1

showed that induction of MI resulted in significant increase in the infarction size from zero to 45.85 ± 2.94 (P < 0.05) while administration of apelin-13 in rats post MI caused significant decrease of the infarction size from 45.85 ± 2.94 to 10.13 ± 0.82 (P < 0.05). Compared to the sham operated group, induction of MI of caused significant increase in the serum level of CK-MB and LDH as they were increased from 3.19 ± 0.62 ng/ml and 24.87 ± 3.48 IU/L to 27.75 ± 1.24 ng/ml and 98.82 ± 3.24 IU/L respectively (P < 0.05). Administration of apelin-13 in rats post MI caused significant decrease of the elevated levels of serum CK-MB and LDH to 4.08 ± 0.75 and 39.59 ± 3.94 respectively (P < 0.05) (table 1 and figure 2). However, there was non-significant differences in CK-MB, LDH and infarction size between the sham operated group (group I) and sham operated + apelin 13 group (group II) (P > 0.05).

Table 1: Effect of Apelin-13 on the infarction size and cardiac enzymes (CK-MB and LDH)

Groups Parameter	Group I (Sham operated)	Group II (Sham operated + Apelin-13)	Group III (MI)	Group IV (MI + Apelin-13)
Infarction size (%)	0	0	45.85 ± 2.94 *	10.13 ± 0.82 $
CK-MB (ng/ml)	3.19 ± 0.62	3.32 ± 0.77	27.75 ± 0.24 *	4.08 ± 0.75 $
LDH (IU/L)	24.87 ± 3.48	25.46 ± 2.16	98.82 ± 3.24 *	39.59 ± 3.94 $

The data were presented as the mean ± standard deviation (SD). Comparisons between groups were analyzed by using one-way Analysis of Variance (ANOVA) followed by post-hoc LSD test. Probability of chance (*P* value) < 0.05 was considered statistically significant.

* Significant difference *vs* the sham operated group
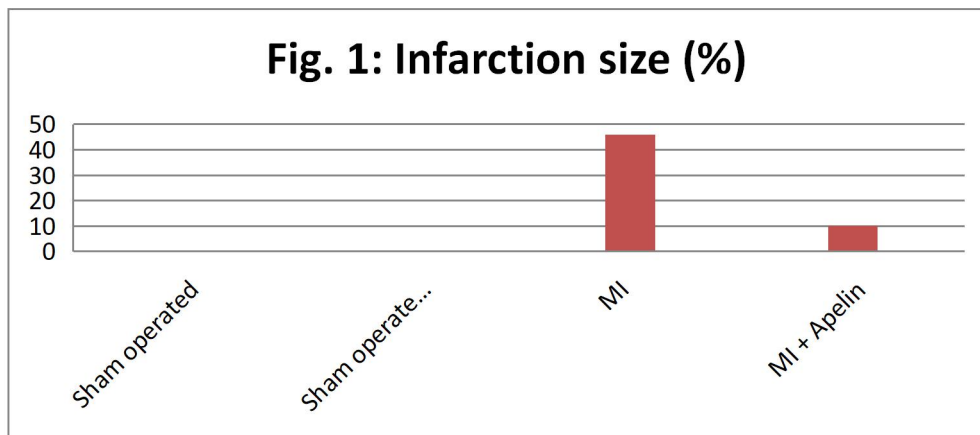$ Significant difference *vs* the MI.
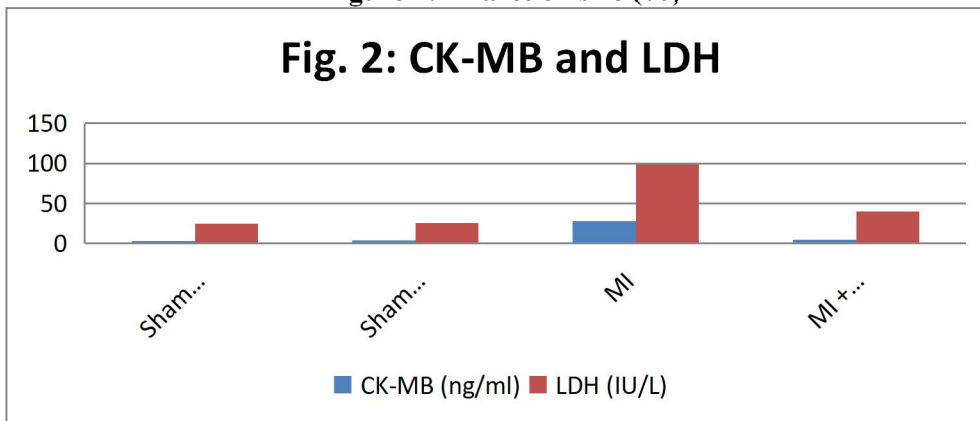

Figure 1: Infarction size (%)

Figure 2: CK-MB and LDH

3.2 Effect of Apelin-13 on circulating angiogenic factors (Table 2, fig. 3, 4 & 5)

There was non-significant differences in circulating angiogenic factors (IGF-1, TGF-β and VEGF between the sham operated group (group I) and sham operated + apelin 13 group (group II) (P > 0.05). Induction of MI caused significant increase in the serum levels of circulating angiogenic factors when compared with sham operated group as IGF-1 was increased from 3.44 ± 0.46 to 9.86 ± 0.92 ng/ml, TGF-β was increased from 6.33 ± 0.88 to 12.67 ± 0.77 ng/ml and VEGF was increased from 145.09 ± 4.80 to 244.50 ± 9.83 pg/ml (P < 0.05) with further increase with apelin treatment in the infarct rats to reach 15.76 ± 0.76, 19.13 ± 0.99 and 338.77 ± 9.56 respectively (P < 0.05).

Table 2: Effect of Apelin-13 on circulating angiogenic factors

Groups / Parameter	Group I (Sham operated)	Group II (Sham operated group + Apelin-13)	Group III (MI)	Group IV (MI + Apelin-13)
IGF-1 (ng/ml)	3.44 ± 0.46	4.24 ± 0.84	9.86 ± 0.92 [*]	15.76 ± 0.76 [$]
TGF-β (ng/ml)	6.33 ± 0.88	7.28 ± 0.48	12.67 ± 0.77 [*]	19.13 ± 0.99 [$]
VEGF (pg/ml)	145.09 ± 4.80	147.12 ± 7.29	244.50 ± 9.83 [*]	338.77 ± 9.56 [$]

The data were presented as the mean ± standard deviation (SD). Comparisons between groups were analyzed by using one-way Analysis of Variance (ANOVA) followed by post-hoc LSD test. Probability of chance (*P* value) < 0.05 was considered statistically significant.

[*] Significant difference *vs* the sham operated group

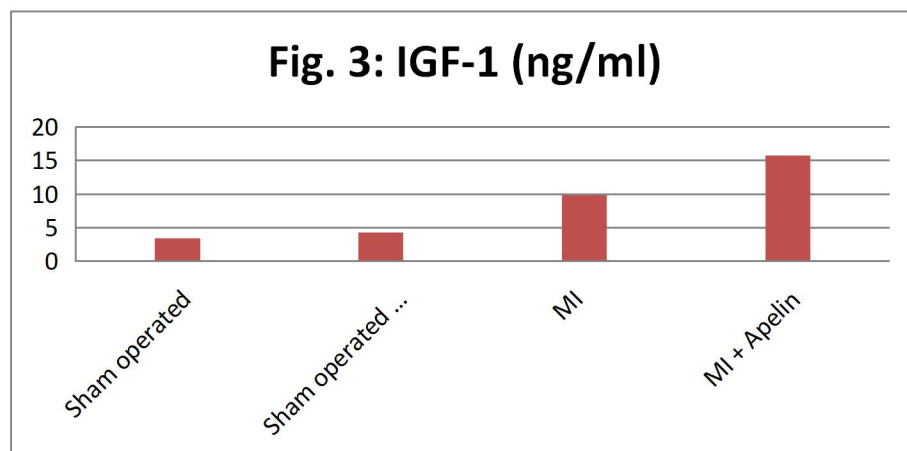[$] Significant difference *vs* the MI group.

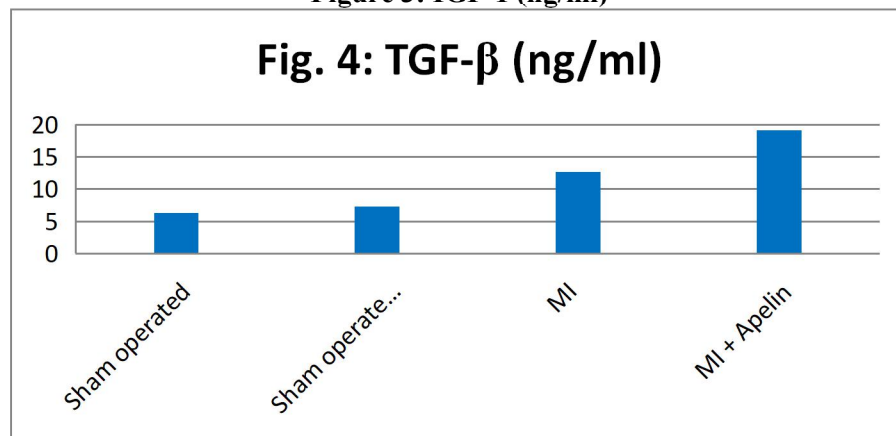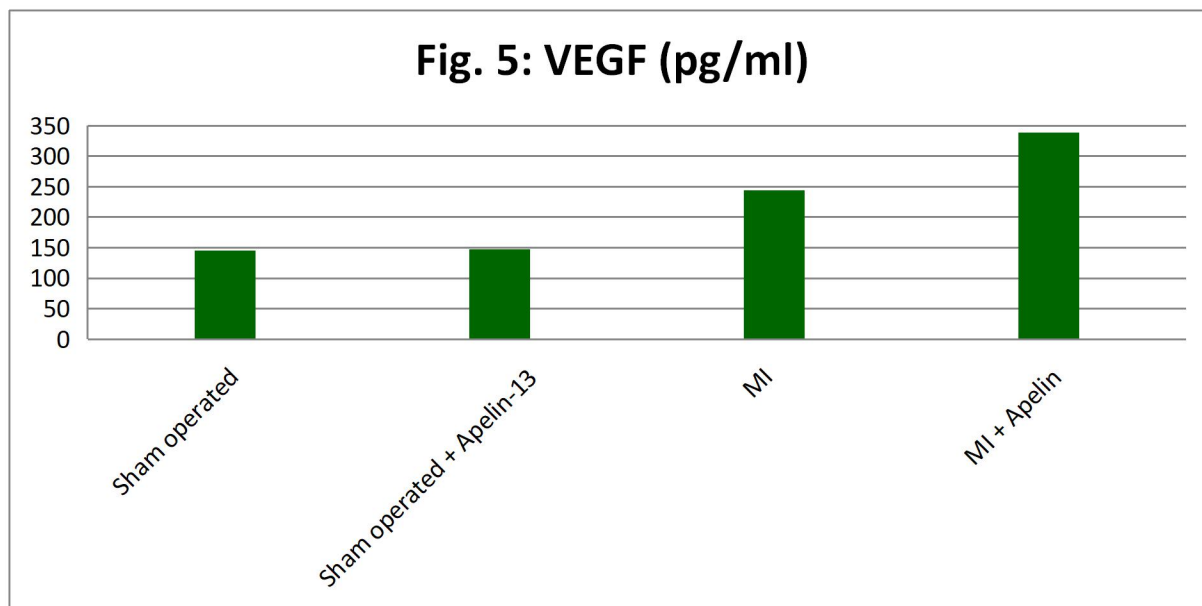
Figure 3: IGF-1 (ng/ml)

Figure 4: TGF-β (ng/ml)

Figure 5: VEGF (pg/ml)

4. Discussion

The present work evaluated the cardioprotective effect of apelin-13 in a rat model of myocardial ischemia, exploring its effects on angiogenesis and functional recovery. In the present study, the left anterior descending artery was ligated to establish acute myocardial ischemia. This led to a significant increase in the infarction size and the myocardial enzymes, including CK-MB and LDH. This result was in agreement to the previous studies [18, 19]. The present work also revealed that circulating levels of angiogenic factors (TGF-β and VEGF) and the proangiogenic growth factor (IGF-1) were significantly increased in the MI group when compared to the sham operated group. Ischemic injury is known to stimulate the release of angiogenic factors derived from the local tissue into the blood circulation [20, 21, 22] and this could be an adaptive response for revascularization and repair of the myocardium to maintain adequate oxygen supply after myocardial ischemia [23]. VEGF is rapidly induced in the ischemic heart in both rodents and humans [12, 24]. *Rabinovsky et al.* pointed that stimulation of angiogenesis by VEGF that occurred after ischemia helped in the regulation of endothelial cell proliferation and migration as well as cell survival [25]. TGF-β also played a major role in angiogenesis by stimulating extracellular matrix production, stabilizing newly formed blood vessels and influencing the expression of other angiogenic factors [26]. The proangiogenic growth factor; IGF-1 promoted angiogenesis by stimulating the release of other angiogenic factors such as VEGF and TGF-β [17].

In acute myocardial ischemia, apelin may have a protective role. The results of the present work revealed that administration of apelin-13 in the MI group led to significant reduction in the infarction size and cardiac biomarkers; CK- MB and LDH. Our results are in congruent to the previous studies which revealed that apelin administration attenuated myocardial injury induced by isoproterenol overdose [27] or left anterior descending coronary artery ligation in rats [15]. In vitro and vivo experimental studies have observed that apelin and apelin receptor gene expression were up-regulated in response to myocardial infarction in cardiac and peripheral tissue [28, 29]. Exogenous apelin infusion improved cardiac contractility, suggesting that the endogenous apelin production was insufficient to activate all the apelin receptors. Therefore, the apelin - apelin receptor system may be induced in ischemia to limit myocardial injury [30, 31]. In mouse hearts, a reduction in infarction size resulting from occlusion of the left descending coronary artery occurred to a greater extent with apelin-13 and to a lesser extent with apelin-36 [15, 32, 33]. In the isolated rat heart, the size of a MI caused by 35 min of occlusion of the left main coronary artery was significantly reduced if exogenous apelin solution was infused for 20 min [34].

Increased angiogenesis and the formation of new blood vessels are critical processes in the restoration of coronary blood flow and in the repair of ischemic injury post MI [35]. The present work also showed that post-infarction treatment with apelin-13 significantly increased the serum levels of angiogenic factors (IGF-1, TGF-β and VEGF). Previous reports showed that apelin increased VEGF/VEGFR2, and angiopoietin-1 (Ang-1)/Tie-2 expression and the density of capillary and arteriole density in the heart [36]. Li et al. found that treatment with apelin-13 resulted in a significant improvement of cardiac function post-MI, promoted myocardial angiogenesis and ameliorated cardiac fibrosis and hypertrophy by a mechanism involving the upregulation of stromal cell derived factor (SDF)-1α/chemokine receptor (CXCR)-4 and homing of vascular progenitor cells (PCs) [37]. An increasing number of evidences demonstrated that angiogenic growth factors have been shown to reduce infarct size, increased vascularity and improve left ventricular ejection fraction in animal models of acute MI [38].

5. Conclusion and Recommendations

Apelin-13 enhanced circulating levels of angiogenic factors; IGF-1, TGF-β and VEGF together with reduction of the enzymes associated with cardiac injury (CK-MB and LDH) and infarction size in rats with induced myocardial infarction. Apelin-13 may enhance neovascularization attributable to the increased presence of angiogenic factors thus it seems to be a novel and efficient therapeutic strategy for myocardial infarction in humans.

List of abbreviations

CK-MB, Creatinine kinase-MB; CXCR, Chemokine receptor; ELISA, enzyme linked immunosorbent assay; IGF-1, Insulin like growth factor one; IHD, Ischemic heart disease; i.p., intraperitoneal; LDH, Lactate dehydrogenase; MI, Myocardial infarction; PC, Progenitor cells; SDF, Stromal cell derived factor; TGF-β, Transforming growth factor beta; TTC, Triphenyl tetrazolium chloride; VEGF, Vascular endothelial growth factor.

Conflict of interest

The author declares that there is no conflict of interest.

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