Irisin: A potentially candidate marker for myocardial infarction

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ABSTRACT

Myocardial infarction (MI) causes energy depletion through imbalance between coronary blood supply and myocardial demand. Irisin produced by the heart reduces ATP production by increasing heat generation. Energy depletion affects irisin concentration in circulation and cardiac tissues, suggesting an association with MI. We examined: (1) irisin expression immunohistochemically in rat heart, skeletal muscle, kidney and liver in isoproterenol (ISO)-induced MI, and (2) serum irisin concentration by ELISA. Rats were randomly allocated into 6 groups ($n = 6$), (i) control, (ii) ISO (1 h), (iii) ISO (2 h), (iv) ISO (4 h), (v) ISO (6 h), and (vi) ISO (24 h), 200 mg ISO in each case. Rats were decapitated and the blood and tissues collected for irisin analysis. Blood was centrifuged at 1792 g for 5 min. Tissues were washed with saline and fixed in 10% formalin for histology. Serum irisin levels gradually decreased from 1 h to 24 h in MI rats compared with controls, the minimum being at 2 h, increasing again after 6 h. Cardiac muscle cells, glomerular, peritubular renal cortical interstitial cells, hepatocytes and liver sinusoidal cells and perimysium, endomysium and nucleoi of skeletal muscle were irisin positive, but its synthesis decreased 1–4 h after MI. At all time-points, irisin increased near myocardial connective tissue, with production in skeletal muscle, liver and kidney recovering after 6 h, although slower than controls. Unique insight into the pathogenesis of MI is shown, and the gradually decrease of serum irisin might be a diagnostic marker for MI.

1. Introduction

Myocardial infarction (MI) is the death of the myocardial tissue that occurs as a result of inadequate coronary blood supply compared to myocardial demand [8]. According to the third monitoring report of the World Health Organization (WHO), 17.3 million people die annually of MI throughout the world [16]. Adenosine triphosphate (ATP) is essential for myocellular viability and normal cardiac functioning, including myofibrillar contraction and ion transport. ATP and phosphocreatine (PCr) are reduced in human MI due to decrease intracellular ATP generation by creatine kinase (CK), due to substrate depletion attributable to myocyte loss [10].

Fibronectin type III domain-containing protein 5 (FNDC5) is proteolytically cleaved and secreted as the hormone peptide, irisin (named after the Greek goddess messenger Iris) [9]. This

peptide converts white adipose tissue (WAT) to brown adipose tissue (BAT), enhancing metabolic uncoupling and hence caloric expenditure upon exercise or temperature. Brown adipose tissue dissipates energy stored in triglycerides as heat via an uncoupling protein 1 (UCP1) [9]. Thus irisin could be a new uncoupling thermogenin peptide that can deplete the body of ATP and increase heat production [5]. Although the role(s) of irisin is/are uncertain (since some inconsistent data has been reported), it has been suggested that irisin might have many physiological functions, such as weight loss, reducing insulin resistance, causing obesity, modulating glucose, and affecting lipid metabolism [7,11,14,19].

Irisin is mainly secreted by heart and skeletal muscle, liver, kidneys, peripheral nerve sheath and dermis and hypodermis of the skin [3,9]. More recently, we reported that cardiac muscle produces more irisin than skeletal muscle. Irisin is mainly produced within heart and skeletal muscle and the total muscle volume, especially heart muscle, which is the best source of irisin, will affect the irisin level [9]. It was interesting to test whether MI has a substantial impact on irisin levels. Isoproterenol (ISO), a β-adrenergic agonist, causes severe stress in the myocardium, resulting in MI due to oxygen deficiency [28]. MI induced by ISO causes similar metabolic and morphological changes in the heart of experimental animals as in man. It is known that ATP and phosphocreatine (PCr) are lowered after MI in man [10]. The increased ATP loss with increased irisin concentration might be related to energy depletion after MI. Some studies showed that impaired cardiac function is detrimental to the kidney (cardiorenal interaction) in clinical [2] and in experimental settings [21]. Myocardial infarction also causes significant abnormal liver functioning [27]. Therefore it is worthwhile checking renal and liver irisin expression in the experimental model of myocardial infarction induced by isoproterenol in rats. However, no study has reported on the fate of irisin in ISO-induced MI. Hence we tested a muscle-derived hormone irisin responsiveness in ISO-induced MI. The first aim was to investigate the expression of irisin in cardiac and skeletal muscle, kidney and liver tissues of rats with ISO-induced MI compared to controls using immunohistochemical analysis. The second aim was to measure irisin serum concentrations by ELISA.

2. Materials and methods

2.1. Animals and experimental design

All protocols of animal experiments were approved (2013-4-69) by the Institutional Animal Ethics Committee (FUIAC) in accordance with the policy of the European convention for the protection of vertebrate animals. All animals were acclimatized for 1 week before starting the experiment, being fed a standard pellet diet and given water ad libitum. Temperature was maintained at $24 \pm 2\,^\circ C$ with alternate 12 h periods of light and dark. Rats (2.5 months old) were divided into 6 groups each of 6 rats. Group I (control), II (1 h), Group III (2 h), group IV (4 h), group V (6 h), and group VI (24 h). Isoproterenol (ISO) hydrochloride (cat. no. I5627) was purchased from Sigma Chemical Co., St. Louis, MO, USA. ISO (200 mg/1000 g body weight) was dissolved in normal saline and injected as a single dose subcutaneously (s.c.) to rats [1] in groups III, IV, V, and VI to induce MI. This dose and route caused significant alterations in biochemical parameters and also moderate necrosis of the heart. At the end of the experimental periods, rats were decapitated under ketamine–HCl (75 mg/kg) and 10 mg/kg xylazine–HCl anesthesia for the estimation of serum and tissue irisin levels. To check whether MI occurred or not, histological sections were prepared and examined by light microscopy to assess gross myocyte injury, as were also biochemical parameters, such as LDH and troponin, which make superior markers of MI. Other details of experimental MI regarding these matters have previously been described [25].

Heart, skeletal muscle, kidneys and liver tissues were resected, cleaned for immunohistochemistry (IHC) and washed in ice-cold saline. Blood samples were divided into 2 aliquots, one for classical biochemical parameters and the other for irisin analysis. Blood was collected into plain biochemical tubes containing 500 KIU aprotinin to protect from proteolysis. Blood was centrifuged at 4000 rpm (1792 × g) at RT for 5 min. Sera were collected and stored at $-80\,^\circ C$ until irisin levels were measured.

2.2. Immunohistochemistry

All tissue samples were immediately placed in 10% neutral buffer formalin. Paraffin embedded 4–5 μm sections were cut and stained with Hematoxylin and Eosin (H&E stain). Sections were examined by light microscopy to assess gross myocyte injury. Irisin status in the heart and other tissues was measured immunohistochemically with avidin–biotin-peroxidase complex (ABC) as per Hsu et al. [15] with minor modification [6]. Briefly, sections were deparaffinized and passed through a graded alcohol series. They were incubated with citrate buffer A solution (pH 6) and heated in a microwave oven (750W; 7 min + 5 min) to recover antigenicity. Sections were allowed to cool for 10 min at room temperature. After cooling, the slides were washed in the PBS (phosphate buffered saline, P4417, Sigma–Aldrich, USA) for 3 × 5 min. After rinsing, endogenous peroxidase activity was depleted by incubation in 3% (v/v) hydrogen peroxide (H_2O_2)-blocking solution (Hydrogen Peroxide Block, TA-125-HP, Lab Vision Corporation, USA) in methanol (5 min at RT). Non-specific staining was blocked with normal goat serum (NGS). Irisin primary antibody was diluted 1/200 ratio (H-067-17, Phoenix Pharmaceuticals, Inc., California, USA), applied and incubated in a humid environment for 60 min at RT. Subsequently, the sections were incubated for 2 h at RT with secondary antibody (biotinylated goat anti-poliyvalent (anti-mouse/rabbit IgG), TP-125-BN, Lab Vision Corporation, USA) for 60 min at room temperature, and washed with PBS for 3 × 5 min, diluted with the same buffer as NGS (1:300). Streptavidin-peroxidase treatment was given (TS-125-HR, Lab Vision Corporation, USA) for 30 min at RT and the slides washed twice for 5 min in PBS and one wash for 5 min in distilled water. Positive reactions were seen by incubating the sections with the chromogen 3-amino-9-ethylcarbazole (AEC) substrate + AEC chromogen (AEC Substrate, TA-015 ve HAS, AEC Chromogen, TA-002-HAC, Lab Vision Corporation, USA). Sections were counterstained with Mayer's stain and hematoxylin, and washed with PBS and distilled water. They were finished through conventional solutions (Large Volume Vision Mount, TA-125-UG, Lab Vision Corporation, USA), dehydrated and mounted with glycerol. Immunostained sections from the cardiac, sketelatal, kidneys and liver tissues were examined with an Olympus BX 50 photomicroscope. Immunohisochemical staining was scored by both its intensity and prevalence on a scale of 0 to +3 (0: absence, +1: weak, +2: medium, +3: strong). Masson's trichrome was also used to stain tissues inorder for histological evaluation. During histological evaluation the following classifications were used (0: absence, +1: weak, +2: medium, +3: strong) noting the increments of inflammatory cells, congestion, fibrosis, edema, disruption of tissue integrity and necrosis. Scoring for each rat was made individually and average values for each group were calculated. The highest score was 18.

2.3. Serological analysis

Serum irisin levels (cat. no. EK-067-52) were measured with a well tested rat-human irisin ELISA (Phoenix Pharmaceuticals, Belmont, California, USA). The lowest detectable concentration of irisin was 9 ng/mL. Intra-assay (within days) and inter-assay (between days) values were 4–6% and 8–10%, respectively. Sample

absorbance at 450 nm was measured with an ELX 800 ELISA reader. Biochemical parameters (troponin, CK-MB, LDH) were measured with an autoanalyzer.

2.4. Statistical analysis

Histological evaluation results were analyzed by one sample Kolmogrov–Smirnov test. Within group comparisons used the analysis of variance (ANOVA) test, followed by Bonferroni for post hoc comparisons. Data were expressed as mean ± SD. The level of significance was taken as $p < 0.05$. SPSS statistical software package, version 21 (SPSS Inc., Chicago, IL), was used for all statistical tests. Spearman's correlation is also used to identify.

3. Results

ISO increased serum creatine kinase muscle-brain fraction (CK-MB) activity [control (7–20 IU/L), experiments (50– ≥150 IU/L)] and cardiac troponin I (cTnI) level [control (0.32–4.1 ng/mL), experiment groups 4.6–6.8 ng/mL) of MI] by 2- to 3-fold and 15- to 20-fold, respectively, compared to the control group. Besides this, histopathological damage confirmed of MI was obtained [(Fig. 1a (control), b (1 h), c (2 h), d (4 h), e (6 h), f (24 h)], as used by other researchers [1,25]. Also Table 1 shows histological damage scores for the whole time period (control, 1 h, 2 h, 4 h, 6 h and 24 h) in rat with ISO-induced MI compared with control.

Immunohistochemical staining showed that cardiac muscle cells have intense (+3) irisin immunoreactivity (black arrows), whereas there was weak (+1) near the connective tissue of heart muscle cells in control rats (red arrows, Fig. 2a). Compared with the control and sham groups, which gave similar results, there was no irisin immunoreactivity differences in the vicinity of connective tissue of heart muscle cells (red arrows) after 1 h of MI, whereas irisin significantly decreased (+1) in heart tissue cells (black arrows, Fig. 2b). At 2 h and 4 h of MI, irisin had markedly decreased (+1) in the heart muscle cells (black arrows). On the other hand, irisin had increased (+3) in the vicinity of connective

tissue of heart muscle cells (red arrows) at these time-points (Fig. 2c and d). By 6 h, decreased irisin (+2) in the heart muscle cells also gradually continued (black arrows), while irisin (+2, red arrows) in the vicinity of connective tissue of heart was raised (Fig. 2e). At 24 h there were no irisin differences (black arrow), the levels having returned to those of normal heart muscle tissues; however, irisin in the vicinity of connective tissue of heart muscle cells (red arrows) remained significantly increased (Fig. 2f).

In skeletal muscle tissues, there was no irisin immunoreactivity of the control group, but interestingly some skeletal muscle cell nuclei were irisin positive (brown arrows). There was also marked irisin immunoreactivity (+3) in the perimysium (red arrows) and endomysium (blue arrows) in the control group (Fig. 3a). Irisin in the other groups was present only in the perimysium of skeletal muscle tissues compared with control groups. Perimysium irisin decreased in all the groups when compared with the controls. Irisin intensities in skeletal muscle tissues were shown as the following: perimysium (red arrow) +2 at 1 h (Fig. 3b); +2 at 2 h (Fig. 3c); (red arrow +1) at 4 (Fig. 3d); (red arrow +1 severity) at 6 h (Fig. 3e); (red arrow +2 severity) at 24 h (Fig. 3f). Irisin immunoreactivity in the perimysium increased beyond 24 h of MI.

Irisin expression was also followed in the kidneys of ISO-induced MI. It was in the glomerular (black arrows) and peritubular interstitial cells (red arrows) in the renal cortex of all the rats. The glomerular region was +1 (Fig. 4a), which was the same as in the control group at 1 h (Fig. 4b). At 2 h and 4 h, irisin had decreased to +2 severity in the glomerular (black arrows) of the renal cortex (Fig. 4c and d). At 6 h and 24 h, irisin had increased to +3 in the glomerular region (black arrows, Fig. 4e and f).

In the liver of rats with ISO-induced M, irisin was found in the hepatocytes (black arrows, +1) and sinusoidal cells (red arrows +3 severity) of the control rats (Fig. 5a). Compared with them, irisin in the sinusoidal cells (black arrows) had decreased in all the groups. Irisin after 1 h of MI was absent from hepatocytes (black arrows), with a severity that has remarkably decreased to +1 in the sinusoidal cells (Fig. 5b). Also, irisin at 2 h, 4 h and 6 h of MI was not seen in the hepatocytes (black arrows) and had decreased to +2 in
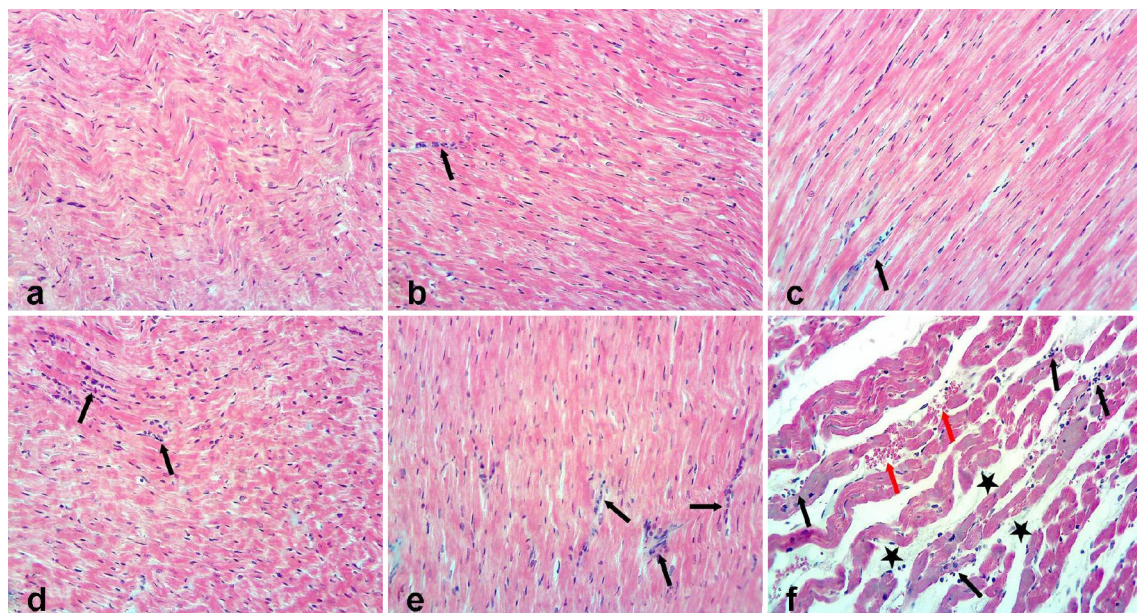

Fig. 1. Histological damage of heart tissues (Masson's trichrome staining) for the whole time period (control, 1 h, 2 h, 4 h, 6 h and 24 h) of observation. Control (a), 1 h [(b) slight increments of inflammatory cells (black arrows)], 2 h [(c) slight increments of inflammatory cells (black arrows)], 4 h [(d) remarkable increments of inflammatory cells (black arrows)], 6 h [(e) remarkable increments of inflammatory cells (black arrows)], 24 h [(f) remarkable increments of inflammatory cells (black arrows), congestion (red arrows); edema and disruption of tissue integrity (black stars)]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Histological damage scores for the whole time period (control, 1 h, 2 h, 4 h, 6 h and 24 h) in rat with ISO-induced MI compared with control.

Groups	Iincrements of inflammatory cells	Congestion	Fibrosis	Edema	Disruption of tissue integrity	Necrosis
Control	0 ± 00	0 ± 00	0 ± 00	0 ± 00	0 ± 00	0 ± 00
1 h	1.33 ± 0.52[a]	0 ± 00	0 ± 00	0 ± 00	0 ± 00	0 ± 00
2 h	1.17 ± 0.45[a]	0 ± 00	0 ± 00	0 ± 00	0 ± 00	0 ± 00
4 h	2.33 ± 0.62[abc]	0 ± 00	0 ± 00	0 ± 00	0 ± 00	0 ± 00
6 h	2.00 ± 0.33[abc]	0 ± 00	0 ± 00	0 ± 00	0 ± 00	0 ± 00
24 h	2.67 ± 0.52[abcd]	2.50 ± 0.55[abcde]	0 ± 00	2.66 ± 0.52[abcde]	2.00 ± 0.89[abcde]	0 ± 00

Control vs. (a) 1 h (MI); (a) 2 h (MI); (abc) 4 h (MI); (abc) 6 h (MI) and (abcde) 24 h (MI); $p < 0.05$.

the sinusoidal cells compared with control tissues (Fig. 5c–e). Irisin immunoreactivity after 24 h was at +1 in hepatocytes and +2 in the sinusoidal cells (Fig. 5f) compared to control liver tissue.

With regard to serum irisin in ISO-induced MI, the control level was 860.3 ng/mL, but had gradually dropped, as indicated here, over the first hour of MI to 418.7 ng/mL, at 2 h 282.7 ng/mL, at 4 h to 323 and at 6 h 406.7 ng/mL. After 24 h, irisin was 484.3 ng/mL (Fig. 6). Irisin measured at 24 h and beyond in MI begin to rise, being ~2-fold lower compared with control serum levels (Fig. 6). There was an inverse relationship between irisin concentration and CK-MB ($r = -0.29$–0.66) and troponin I ($r = -0.33$–0.72) when comapred control after MI.

4. Discussion

MI has been the most common cause of death in the world, and heart muscle death follows with scarring without cardiomyocyte regrowth because of lack of oxygen. Irisin induces heat production when energy molecules (fats and glucose) are without an increase in ATP production due to its uncoupling properties. In ISO-induced MI, serum irisin was gradually decreased compared to the control group. Dai et al. [12] also reported another peptide, nesfatin-1, of which the plasma levels were significantly lower in acute myocardial infarction (AMI) group than in the stable angina pectoris (SAP)

group or control group (0.91 ± 0.08 ng/mL vs. 0.98 ± 0.19 ng/mL and 1.09 ± 0.39 ng/mL, respectively, $p < 0.05$). Why the serum irisin level decreases in rats with this treatment, may be due to the following explanations. MI is characterized by oxygen deficiency, with 30 min of ischemia reducing myocardial ATP by 50%, while restoration of aerobic conditions led to ATP increasing to only 60% of control levels [22]. High irisin concentration produces a loss of ATP because of its uncoupling properties [9], producing a progressive decrease in serum and tissue irisin level as a means of protecting myocardial cells by saving energy that would otherwise have to be given to an increased energy supply to ischemia mycardiocytes, which is achieved by inhibiting ATP loss. When there is more irisin, its uncoupling properties lead to greater ATP loss and greater heat production [9]. We assumed that if irisin level is not decreased in MI, myocardial and other cells would experience more damage. Locally produced heat due to irisin might accelerate to some extent the rate of biochemical reactions.

Another significant interest is that heart and skeletal muscle, liver and kidney tissue irisin levels decreased in the presence of MI compared to the control group. MI causes a depletion of ATP [22]; therefore theoretically, cardiac myocytes and these other tissue cells have limited energy expenditure which help to keep them alive. Heart tissue is the one of the main sources of irisin with other sources [5]. Therefore in order to control energy homeostasis, irisin
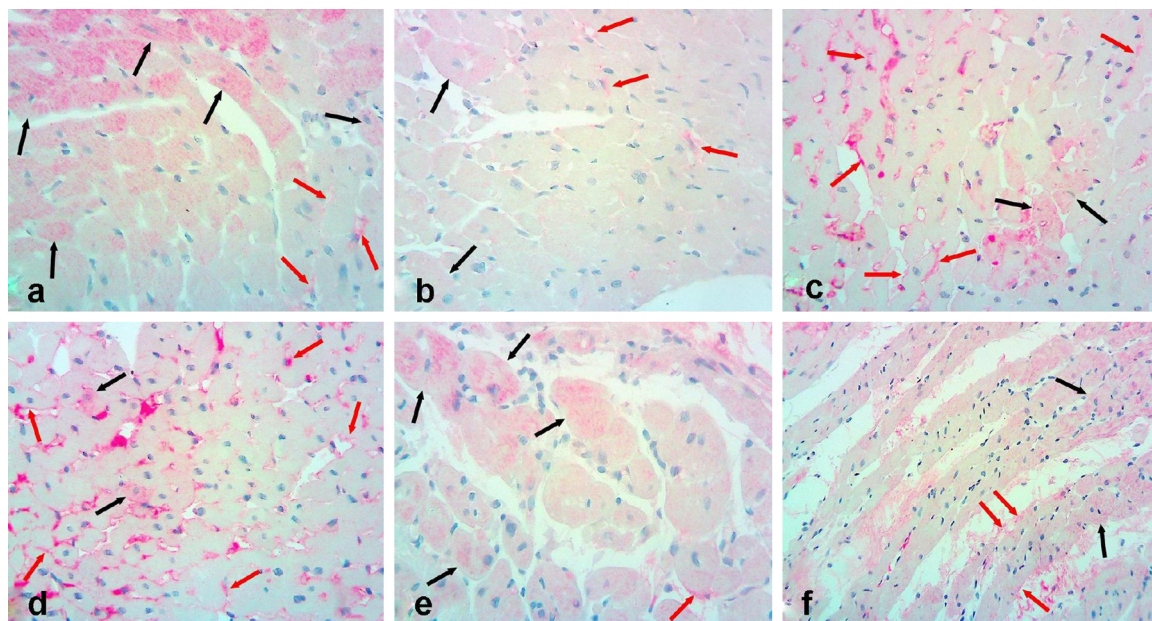

Fig. 2. Immunohistochemical staining of irisin in the cardiac tissues in rat with ISO-induced MI compared with control. Control (a), 1 h of MI (b), 2 h (c), 4 h (d), 6 h (e), 24 h (f). Irisin immunoreactivity in tissues is colored red. Control (a), cardiac muscle cells strong (+3) irisin immunoreactivity (black arrows), weak immunoreactivity (+1) near the connective tissue of heart muscle cells (red arrows). 1 h of MI (b), weak immunoreactivity (+1) in the vicinity of connective tissue of heart muscle cells (red arrows), decreased irisin immunoreactivity (+1) in heart tissue cells (black arrows). At 2 (c) and 4 h (d) of MI, irisin markedly decreased (+1) in the heart muscle cells (black arrows), but irisin immunoreactivity increased (+3) in the vicinity of connective tissue of heart muscle cells (red arrows). 6 h (e), irisin immunoreactivity (+2) in the heart muscle cells (black arrows) and in the vicinity of connective tissue of heart irisin (+2, red arrows). 24 h (f), when compared with control, no irisin immunoreactivity differences (black arrow), however, irisin immunoreactivity (+3) in the vicinity of connective tissue of heart muscle cells (red arrows) increased. Mag. 400×. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
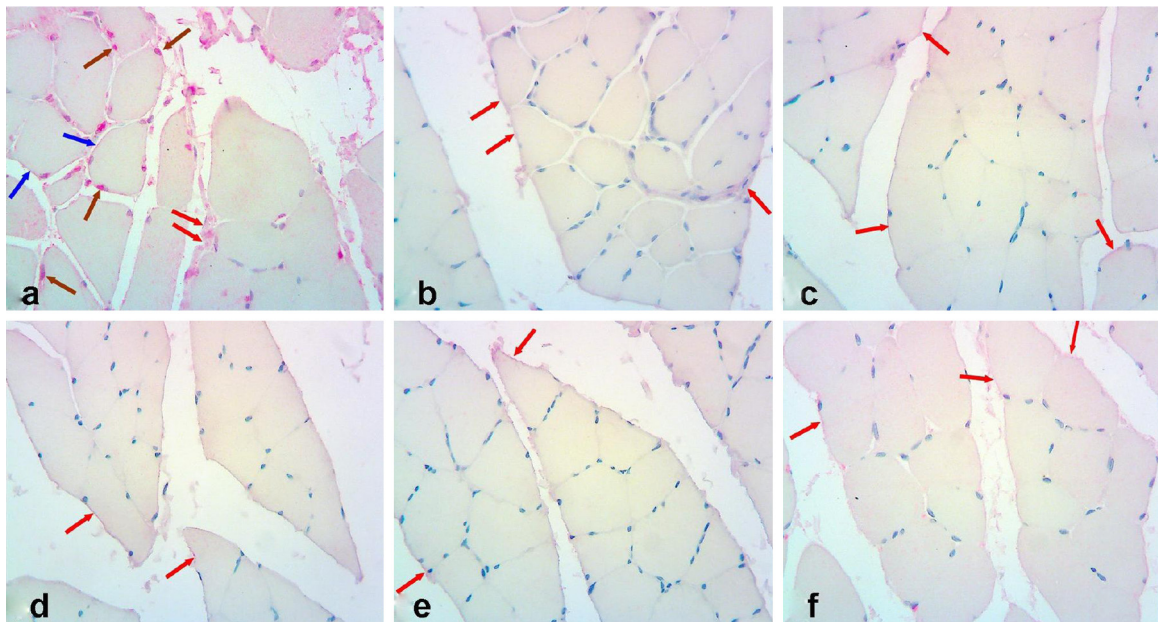
Fig. 3. Immunohistochemical staining of irisin in the skeletal muscle tissues in rat with ISO-induced MI compared with control. Control [(a) muscle cell nuclei (brown arrows); the perimysium (red arrows) and endomysium (blue arrows) irisin immunoreactive parts], 1 h [(b) only irisin immunoreactivity in the perimysium (red arrows)], 2 h [(c) only irisin immunoreactivity in the perimysium (red arrows)], 4 h [(d) only irisin immunoreactivity in the perimysium (red arrows)], 6 h [(e) only irisin immunoreactivity in the perimysium (red arrows)], 24 h [(f) only irisin immunoreactivity in the perimysium (red arrows)]. Perimysium irisin decreased in all the groups compared with the controls. Mag. 400×. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

producing cells in the heart are switched off in the presence of MI. Here significant decreases of irisin in the heart and other tissues in the presence of MI might save energy for tissues where there is depleted ATP and increased energy demands – if tissues did not react in this way, more irisin would lead to greater energy depletion and the more heat production. Thus heart tissues starved of energy would become necrotic more quickly (Table 1). Therefore by blocking de novo irisin production, we assume that heart tissues will be saved and protected from damage to some extent.

Heart connective tissues in rats with ISO-induced MI gradually increased from throughout a day compared with their corresponding controls. Previously Ross and Glomset [23] postulate that the lesions in atherosclerosis arise as a result of some form of "injury" to the arterial endothelium. This injury results in alteration in endothelial cell–cell attachment or endothelial cell-connective tissue attachment; this leads to adherence and aggregation of platelets at the sites of focal injury. The process of the release of a mitogenic factor in their contents results in the focal intimal
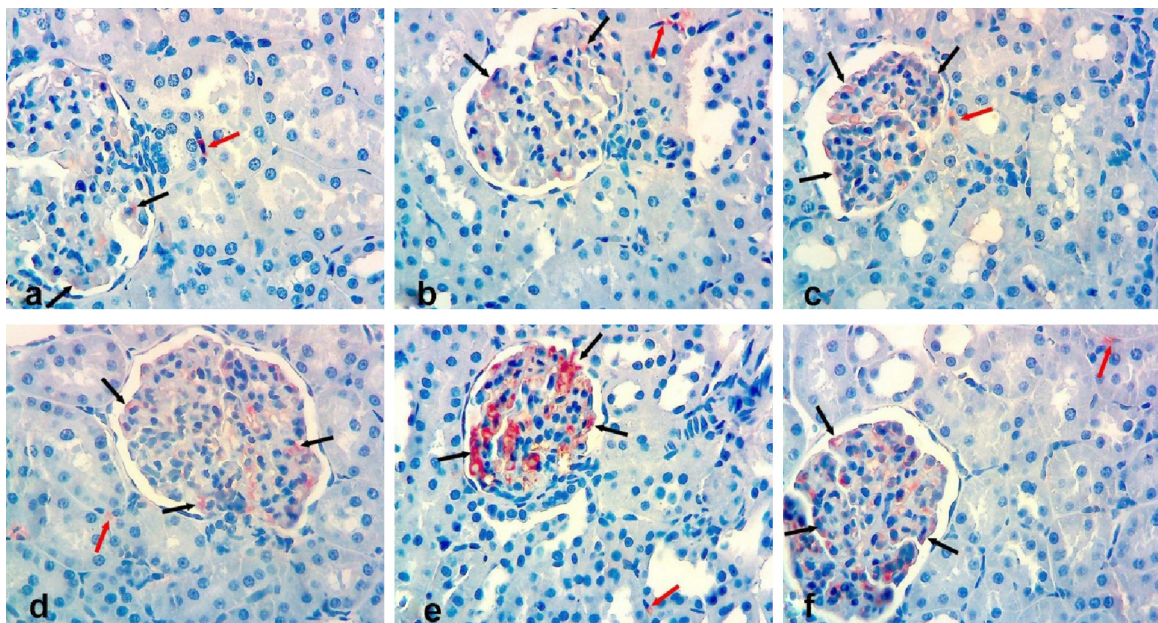

Fig. 4. Immunohistochemical staining of irisin in the kidneys tissues in rat with ISO-induced MI compared with control. Control (a), 1 h (b), 2 h (c), 4 h (d), 6 h (e), 24 h (f). It was in the glomerular (black arrows) and peritubular interstitial cells (red arrows) in the renal cortex of all the rats. At 2 h and 4 h, irisin had decreased to +2 severity in the glomerular (black arrows) of the renal cortex (c and d). At 6 h and 24 h, irisin had increased to +3 in the glomerular region (black arrows, e and f). Mag. 400×. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
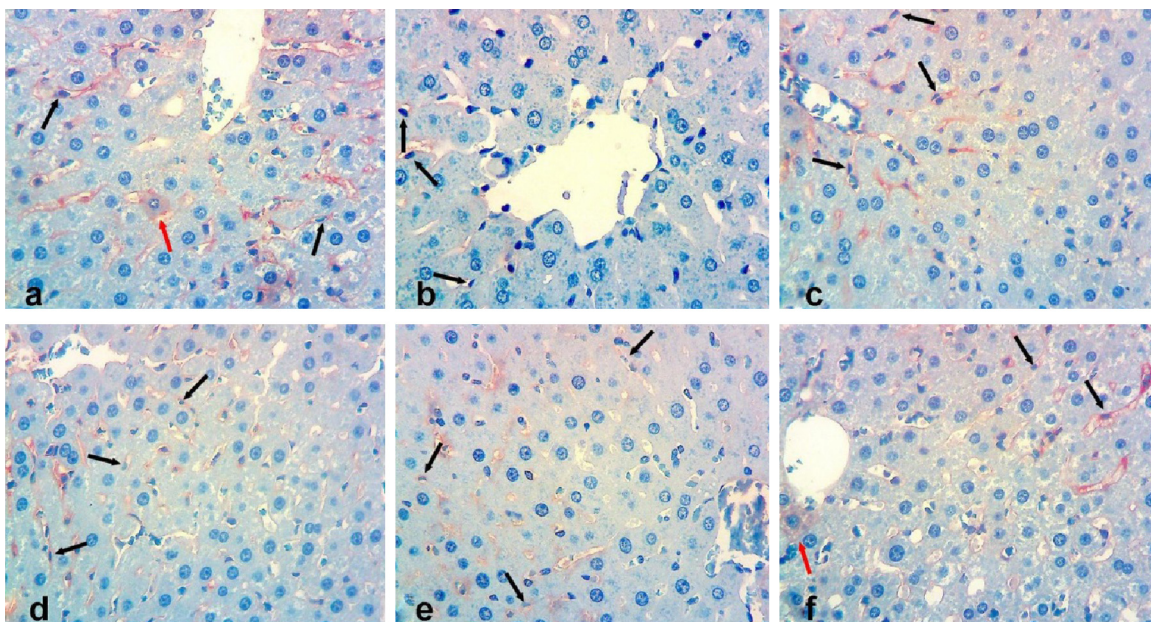
Fig. 5. Immunohistochemical staining of irisin in the liver tissues in rat with ISO-induced MI compared with control. Control (a), 1 h (b), 2 h (c), 4 h (d), 6 h (e), 24 h (f). Irisin was found in the hepatocytes (black arrows, +1) and sinusoidal cells (red arrows +3 severity) of the control rats (a). Compared with them, irisin in the sinusoidal cells (black arrows) had decreased in all the groups (b–f). Mag. 400×. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
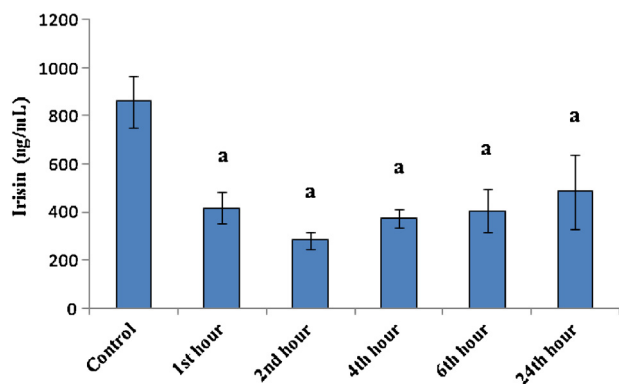

Fig. 6. Serum irisin levels in rat with ISO-induced MI compared with control. Control, 1 h, 2 h, 4 h, 6 h and 24 h Other details are given in the text. Serum irisin levels are statistically lower compared with control (a: $p < 0.05$). Each data point is the average of 3–6 individual rats.

proliferation of smooth muscle cells. This proliferation is accompanied by the formation of large amount of new connective tissue matrix proteins. Based on our results, irisin might be one of them, because we have previously shown that connective tissue is one of the major irisin producing tissues [5]. Increased new connective tissue means increased irisin level, which means increased ATP consumption. Thus, decrease of energy molecules in tissues might cause less tissue injury and leave scar tissues, and the late healing of tissues. It is assumed that a normal physiological irisin concentration might be an important factor in normal tissue healing.

We have also confirmed our previous results that showed that irisin is present in cardiac muscle cells, connective tissue in the vicinity of the heart, glomerular and peritubular interstitial cells of the renal cortex, hepatocytes and sinusoidal cells of the liver, and perimysium of skeletal muscle tissues [5]. Besides perimysium skeletal muscle tissues (previously published), endomisyum and nuclei of skeletal muscle tissues were irisin positive [5]. Previously it had been shown that some other peptides are produced in the cardiac muscle (e.g., BNP, ANP [13] and salusins [4]), skeletal muscle

(e.g. interleukin-6 and some other myokins (e.g. irisin) [20], ghrelin [24], liver (e.g. adropin [6] and kidney tissues (adropin [17]), which (liver and kidneys) are the mostly affected organs with MI. Locally produced irisin in these tissues might have paracrine and autocrine function as do other locally produced peptides [4,6,17,20,24]. We also tentatively suggest that irisin might have an ATP sensor role in local tissues, depending on their energy requirement. If ATP was unnecessary in the cell, turning on its expression, will result in energy being released as heat in physiological irisin levels [9]; if ATP is required in the local tissues, irisin production turns it down in the local tissues. The more irisin means greater heat without ATP production, and vice versa, the less irisin means less heat production, making ATP from energy supply molecules such as fat and glucose [9]. Also, MI alters some peptide hormones in the tissue and circulation; others have reported an increment [26], and some a decrease [12]. We are also currently unable to compare our results with others regarding serum and tissue concentration, since, as far as we know, this was first report about irisin concentration decreases in MI.

4.1. Conclusion

Irisin intensively increases in the connective tissue in the vicinity of the heart muscle cells in MI. Connective tissue is a major irisin producer. Increased connective tissue leads to increased irisin, a loss of ATP in the local tissues, and loss of ATP in the heart tissue might be responsible for the scar tissue following MI because of energy depletion via irisin heat production. Irisin in MI is considered, at least in part, as a cellular 'energy sensor' blocking white adipose tissue to brown adipose tissue, resulting in decreased metabolic thermogenesis. It might also contribute to the decrease of heat and sweat. Along with the regulation of energy balance, we also think that decreased irisin as an uncoupling agent in the circulation inhibits ATP consumption and thus might maintain cell life and inhibit to some extent necrosis. These findings along with previous data of others lead us to think that there might be a link between MI [18] and circulated irisin concentration that increases after indurance exercise. In human subjects, there is also an

association between saliva/serum irisin concentration and MI as in the animal experimental setting (manuscript in preparation). Furthermore, the data are promising that serum irisin might reflect MI and is a biological marker of MI.

Conflict of interest

The authors declare that they have no conflict of interest.

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