Effect of Myocardial Infarction on Spleen and Kidney of Isoprenalin-induced Myocardial Injury Rat Model

Shafreena Shaukat Ali, Nur Hafiqah Rozalei, Siti Fatimah Azaharah Mohamed, Yap Wei Boon, Satirah Zainalabidin

Abstract

Myocardial infarction (MI)-associated inflammation is crucial for post-infarct healing. However, unregulated inflammation may lead to unnecessary systemic inflammation and affect other organs. This study investigated the effects of MI on spleen and kidneys over early time-points (2 and 7 days) in isoprenalin-induced MI rat model. MI was evident by elevated levels of cardiac injury marker, troponin T, and lactate dehydrogenase (LDH). MI rats showed significant upregulation of myeloperoxidase (MPO) activity at day-2 post infarct while kidney MPO activity remained unaffected. Kidney function test revealed only slightly increased plasma urea at day-2 post MI with no changes in plasma creatine at both time-points. Histological observation on the spleen showed disorganization of spleen structure at day-2 post MI while the kidney structures were preserved at both time-points. In conclusion, although MI-associated damages in the spleen were seen at day-2 post infarct, it had no impact on the kidney structures at both time-points.

Keywords: Inflammation, isoprenalin, kidney, myocardial infarction, spleen

Abstrak


Kata kunci: Inflamasi, isoprenalin, ginjal, infarksi miokardium, limpa

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1.0 INTRODUCTION

Globally, ischemic diseases, particularly myocardial infarction (MI), contribute to the highest number of morbidity and mortality. Despite advancement in healthcare settings, MI is still the main culprit of premature death globally, and remains a major concern as it is a great burden to public health and the economy [1]. In Malaysia alone, MI is responsible for 13.2% of total deaths[2]. Myocardial injury due to infarction is defined as myocardial cell death resulting from an imbalance between supply and demand of blood and oxygen in the heart [3].

Various MI models such as coronary artery ligation and isoprenaline (ISO)-induced models have been established to understand the pathophysiology, and complications of MI. Coronary artery ligation model requires ligation of left descending artery to stop blood supply to myocardium. However, this invasive method has drawbacks as it requires high surgical and technical skills, besides it also has a low percentage of surgery success. Furthermore, invasive procedures in coronary artery ligation contribute to a higher mortality rate compared to isoprenaline-induced MI model [4]. Isoprenaline, a synthetic catecholamine, and nonspecific beta-adrenergic receptor agonist, is able to inflict myocardial injury followed by necrosis of the heart muscles [5]. Compared to other models, ISO-induced model has a lower mortality rate, and technique simplicity for induction of MI [6]. Several lines of evidence showed that ISO is able to cause myocardial aberration which is similar to those seen in humans clinically [7, 8, 9]. Therefore, ISO-induced MI models were used in this study.

It is well known that MI triggers local inflammatory reactions, where inflammation is crucial for repair processes in injury sites [10, 11, 12, 13]. However, prolonged local inflammation may lead to unnecessary systemic inflammatory reactions. Previous studies have shown that MI promoted recruitment of leukocytes from bone marrow and spleen to the site of injury [14, 15]. This led to increased circulating leukocytes, and indirectly triggered unnecessary inflammation in other organs such as kidneys [16, 17]. This was evident with infiltration of leukocytes into kidney glomeruli within 24 hours post MI [16] in a ligation model. As evidence of systemic inflammation involved in ISO-induced MI models is still lacking, this study investigated the effects of systemic inflammation following myocardial injury to vital organs such as kidney and spleen in ISO-induced MI rats.

2.0 METHODOLOGY

2.1 Animals

A total of twenty-four Wistar male rats (200-300g; 7-8 weeks) were obtained from the Laboratory Animal Resource Unit, Faculty of Medicine, Universiti Kebangsaan Malaysia. The animals were acclimatized for one week before the study, and fed with normal pellet and ad libitum access to water. The animals were housed in standard cages, and maintained under standard laboratory conditions with ambient room temperature, and lighting (12–12 hour light/dark cycle). The experimental protocol was subjected to approval by the Ethics Committee of Animal Use, Universiti Kebangsaan Malaysia (FSK/2016/SATIRAH/23-OCT./812-November2016-November2017).

2.2 Study Design

The rats were randomly divided into two groups (n=6): Control and MI. The MI group was injected subcutaneously with isoprenaline hydrochloride (ISO) (Tokyo Chemical Industry, Japan) dissolved in normal saline at 85 mg/kg [18], while the control group received subcutaneous injection of normal saline. Both treatments were carried out for two consecutive days with a 24-hour interval. The rats were then sacrificed at different time-points, i.e., 2 and 7 days after the last injection of ISO. They were anesthetized with 1 g/kg urethane (Sigma Aldrich, US), and sacrificed by cervical dislocation. Blood samples were collected in heparin tubes, separated at 3500 rpm for 5 min, and stored at -80°C until further analysis. The spleen and kidneys were excised immediately, and processed for biochemical assay, and histopathology. Prior to biochemical analyses, the spleen and kidneys were homogenized with ice-cold Tris-buffer (10 mM) using tissue homogenizer. The tissue homogenates (3 %, w/v) were then centrifuged at 8,000 rpm for 20 minutes at 4°C, and kept at -80°C until further analysis.

2.3 Estimation of Plasma Troponin T

Plasma levels of troponin-T were quantitatively measured using commercial ELISA kit (Elabscience Biotechnology Co., Ltd). Briefly, 100 µL of standard or plasma samples were added into microplate wells pre-coated with specific antibodies, and incubated at 37°C for 90 minutes. Excessive solution was decanted, and the wells were washed three times with PBS. Then, 100 µL of biotinylated specific antibody (1:100 dilutions) was added, and incubation was done at 37°C for 1 hour, followed by 30-minute incubation with 100 µl avidin-conjugated horseradish peroxidase (HRP) (1:100 dilutions) at 37°C. Excessive solution was decanted, and the wells were washed three times as described previously. Substrate solution (90 µl) was added to each well to allow enzyme-
substrate reaction. Enzyme-substrate reaction was terminated with 50 μL stop solution. The microplate was read spectrophotometrically at 450 nm.

2.4 Estimation of Plasma Lactate Dehydrogenase

Plasma lactate dehydrogenase (LDH) activity was measured as previously described with slight modifications [19]. Briefly, 10 μL of plasma samples were added into microplate wells followed by 240 μL of PBS. Then, 10 μL of nicotinamide adenine dinucleotide (NADH) solution (2.5 mg/ml) was added, and the plate was incubated at 25°C for 20 minutes. Next, 10 μL of sodium pyruvate solution (2.5 mg/ml) was added. LDH activity was measured 5 times at 340 nm for 5 minutes with 1-minute intervals.

2.5 Estimation of Plasma Creatinine

Plasma creatinine was measured as follows. Briefly, 0.5 mL of plasma samples was added followed by 4 mL of tungstic acid. The mixture was then centrifuged at 3000 rpm for 5 minutes. Then, 250 μL of supernatant was transferred into the microplate wells, followed by 100 μL of picric acid (0.036 M), and 50 μL of NaOH (1.4 M). The mixture was incubated in the dark at room temperature for 15 minutes, and read at 520 nm.

2.6 Estimation of Plasma Blood Urea Nitrogen

For estimation of plasma blood urea nitrogen, about 0.5 mL of plasma samples was added to 4.6 mL of isotonic solution, and 200 μL of NaOH (0.5 M). The mixture was centrifuged at 3500 rpm for 5 minutes. Then, 250 μL of supernatant was aliquoted, and added to 400 μL of diacetyl monoxime solution, followed by 200 μL of phosphoric acid. The mixture was boiled at 100°C for 30 minutes and subsequently read at 480 nm.

2.7 Estimation of Myeloperoxidase (MPO) Activity

MPO activity in the spleen and kidneys was measured using hydrogen peroxide, and o-dianisidine dihydrochloride. Spleen and kidney tissue homogenates were prepared as recommended by Kim et al. [20] and Ramalingam et al. [21]. About 7 μL of tissue homogenates were added into the microwells, followed by 200 μL of o-dianisidine, then measured immediately thrice at 450 nm with 30 second intervals between each reading.

2.8 Histopathological Evaluation

Small portions of kidney and spleen tissues were fixed with 10% neutral-buffered formalin, and embedded into paraffin blocks. Tissues were sectioned into 4-μm thickness using rotary microtome, and stained with hematoxylin and eosin for microscopic observation.

2.9 Statistical Analysis

Data was presented as mean ± SEM. Unpaired student t-test was used to compare parametric data between groups. All statistical analyses were performed using GraphPad Prism 5. A two-tailed P-value of 0.05 was considered statistically significant.

3.0 RESULTS AND DISCUSSION

3.1 General Characteristics and Cardiac Injury Marker

Table 1 shows a summary of the results on general characteristics, and cardiac injury markers of control and MI groups at both time-points. BW, food intake, and water intake of MI group were not affected at both time-points. Troponin-T level was significantly elevated (p<0.05) in the MI group for both time-points, indicating myocardial injury. Similarly, elevated LDH level was observed at both time-points although the increase was not significant in comparison to troponin-T. About 10% and 13.3% mortalities were recorded at day-2, and -7 post MI, respectively. There were no mortalities recorded in the control group for both time-points.

Isoprenaline, a non-specific, beta-adrenergic receptor agonist, and synthetic catecholamines, has been reported to produce myocardial aberrations in rat model of MI. Following ISO-induced MI, injury markers such as troponin T, and LDH leaked from myocardium to circulation due to disrupted cellular membrane integrity [22, 23]. Consistent with other studies, elevation of troponin T levels was evident in this study at day-2 and -7 post MI. Plasma level of troponin T is directly proportional to the degree of necrotic lesions present in the myocardium, and is thus a specific marker of myocardial damage. Troponin is generally released as early as 2-3 hours after the onset of MI and peaked at 24-48 hours, and may persist up to 14 days [24]. In light of this, the rise of troponin T level is a reliable indicator of myocardial tissue damage [25]. However, plasma levels of LDH did not increase significantly at both time-points. This could be due to short persistence of LDH in the circulation following the occurrence of MI where LDH level normally peaks for 2-3 days, and starts to normalize to baseline at day-7 [26].
Table 1 General characteristics and cardiac injury markers

<table>
<thead>
<tr>
<th></th>
<th>2 Days</th>
<th>7 Days</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>MI</td>
</tr>
<tr>
<td>Body weight (%)</td>
<td>54.5±7.2</td>
<td>50.0±7.0</td>
</tr>
<tr>
<td>Food intake (g)</td>
<td>20.8±0.4</td>
<td>18.1±0.4</td>
</tr>
<tr>
<td>Water intake (ml)</td>
<td>26.4±0.4</td>
<td>35.6±12.1</td>
</tr>
<tr>
<td>Troponin T (pg/ml)</td>
<td>17.7±1.3</td>
<td>33.6±7.2*</td>
</tr>
<tr>
<td>LDH (U/ml)</td>
<td>0.02±0.01</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>Mortality rate (%)</td>
<td>ND</td>
<td>10</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM for n=6 per group. *p<0.05 in relative to Control

3.2 MPO Activity of the Spleen and Kidney in MI Rats

MI rats showed an upward trend of spleen MPO activity (see Figure 1) at both time-points especially at day-2 post MI induction. Nevertheless, there were no significant differences in the MPO activity of kidneys (see Figure 2) at day-2, and -7 post MI, thereby suggesting that the kidneys were not affected.

Neutrophils infiltration in acute inflammation ensures healing due to myocardial injury [11]. Myeloperoxidase (MPO), found in abundance in neutrophils, has been used to estimate the degree of neutrophils infiltration directly [27, 28, 29]. Our findings showed a significant increase in MPO activity in the spleen of MI rats at day-2 post MI. However, MPO activity at day-7 post MI was not greatly affected. According to Swirski et al. [14], leukocyte proliferation in the spleen within 24 hours post MI recruits neutrophils to myocardial injury site in order to initiate the healing processes. It is therefore speculated that the resolved inflammation at day-7 post MI was responsible for the insignificant elevation of MPO activity in the spleen. This is parallel to the findings of Schloss et al. [29] in which neutrophil infiltration was reported to decline at 72 hours post MI.

Figure 1 MPO activity of spleen in post MI model. Measurement of MPO activity in the spleen. MPO activity was elevated in the spleen in response to MI. Values are presented as mean ± SEM for n=6 per group. *p < 0.05 relative to Control

3.3 Kidney Function Test of MI Rats

The test results of kidney function showed no significant differences in plasma creatinine levels (see Figure 3) between MI, and control rats at both time-points. Plasma urea levels (see Figure 4) slightly increased in MI rats at day-2 post MI, but it was not statistically significant. ISO-induced MI also had no effect on plasma urea levels in both rat groups at day-7 post MI.

A few studies reported evidence of kidney injury following MI [17, 30]. According to Lekawanvijit et al. [17], MI rats exhibited kidney damage at day-7 of coronary ligation-mediated MI. Hypoperfusion in post MI settings was able to induce ischemia injury in the kidneys, which then triggered local inflammation. Nevertheless, our findings showed the contrary in which the kidneys remained unaffected at both time-points post MI where plasma levels of creatinine, and urea remained unaffected at both time-points.

Figure 2 MPO activity of kidney in post MI model. Measurement of MPO activity in the kidneys. MPO activity of the kidneys was not affected by MI. Values are presented as mean ± SEM for n=6 per group. *p < 0.05 relative to Control

Figure 3 Kidney function test in post MI model. Measurement of plasma creatinine levels. There was no significant elevation in plasma creatinine levels in response to MI. Values are presented as mean ± SEM for n=6 per group
Figure 4 Kidney function test in post MI model. Measurement of plasma urea levels. There was no significant elevation in plasma urea levels in response to MI. Values are presented as mean ± SEM for n=6 per group.

3.4 Histological Examination of Spleen Kidney of MI Rats

Histological examination revealed no structural changes in kidney tissues (see Figures 5a-b) of MI rats compared to healthy control rats at both time-points. The observation showed preserved renal corpuscles with glomeruli in both groups. The anatomical structures of the kidneys of MI rats were comparable to the kidneys of healthy control rats. Spleen tissues (see Figures 6a-4b) of healthy control rats showed preserved structures germinal centres. Red pulp and white pulp areas were readily distinguishable. However, in MI spleen tissues, slightly disorganized germinal centres were observed beside poorly distinguishable red pulp, and white pulp areas. This could be due to increased proliferation of leukocytes in the germinal centres which resulted in the expansion of germinal centres.

Figure 5 Histology of kidney in post MI model. Representative images of H&E-stained kidney sections at day-2 (a), and -7 (b) post MI; 40x magnification; scale bar 50µm. Preserved structures of renal corpuscles with glomeruli (G) were observed in both control and MI tissues.

Figure 6 Histology of spleen in post MI model. Representative images of H&E-stained spleen sections at day -2 (a), and -7 (b) post MI; 40x magnification; scale bar 50µm. Preserved structures of germinal centres (GC) and distinguishable red pulp (RP) and white pulp (WP) areas were observed in control group. MI spleen tissues exhibited slight disorganization of GC, RP, and WP areas.

4.0 CONCLUSION

In summary, this study provides evidence on the effects of MI on the spleen, and kidneys of ISO-induced rats at day-2, and -7 post MI. Following MI, the spleen was only affected at day-2, but not at day-7 post MI. This was well characterized by biochemical, and histological analyses. Following induction of MI, it has been proven that the kidneys of the MI rats were unaffected based on biochemical and histological observations.

Acknowledgement

This research was fully supported by Malaysian Toray Science Foundation (MTSF) under the Grant Code NN-2016-082.

References


