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Thrombolytic Therapy Up-regulates Inflammatory Mediators Compared to Percutaneous Coronary Intervention (PCI)

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ABSTRACT

The important role of reperfusion therapies in the treatment of acute myocardial infarction is well documented. However, reperfusion therapies can initiate inflammatory response and may damage the myocardium. The purpose of current study was to compare the effects of percutaneous coronary intervention and thrombolytic therapy on inflammatory markers in the setting of ST elevation myocardial infarction (STEMI).

Eighty three patients with STEMI were enrolled in this study. 40 patients underwent percutaneous coronary intervention **(**PCI), and 43 patients received streptokinase (1.5 million IU) as a main medical reperfusion therapy. Monocyte expression of Toll-like receptor 4 (TLR4), serum levels of TNF-α and IL-1β, red cell distribution width (RDW) and C- reactive protein (CRP) were compared between groups at admission time, two hours and four hours after termination of treatment. $p<0.05$ was considered as statistically significant for all tests.

Compared to baseline, both treatments increased monocyte expression of TLR4, serum levels of cytokines and CRP. Compared to PCI, medical reperfusion therapy significantly raised both monocyte expression of TLR4 (39.8±4.7 % vs 49.1±3.6 %, $p<0.01$), and serum levels of TNF-α (13.2±3.7 pg/ml vs 25.1±2.6 pg/ml $p<0.05$). No effect was seen on RDW levels. Moreover, medical reperfusion therapy caused significant rise in CRP levels ($p<0.01$).

The present study demonstrates that thrombolytic therapy is associated with higher inflammatory responses compared to PCI. Our findings suggest that thrombolytic therapy may increase the likelihood of detrimental effects of reperfusion therapy on the myocardium.

Keywords: Inflammation; Percutaneous coronary interventions; Reperfusion therapy; ST elevation myocardial infarction; Toll-like receptor 4

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INTRODUCTION

Coronary artery disease (CAD) is the leading cause of mortality in the world.[1] Atherosclerosis, as an underlying mechanism of CAD, is a well-defined inflammatory process .Immune cells have pivotal role in formation, progression and disruption of the atherosclerotic plaque.[2] Severe occlusion of the coronary vessels is usually present in patients with acute myocardial infarction (AMI). Restoration of blood flow to the ischemic heart may cause inflammatory damages to the myocardium known as ischemia/reperfusion (I/R) injury.[3,4] Emerging evidence emphasizes on the role of toll-like receptor 4 (TLR4) in the ischemic heart disease (IHD) and also I/R injury. Patients with IHD have usually high expression of TLR4 on the surface of $CD14^+$ monocytes.[5-7] TLR4 activation by heat shock proteins 60 and 70, minimally modified LDL and oxidized LDL leads to translocation of nuclear factor kappa B (NF-κB) and finally production of pro-inflammatory cytokines.[8] Cytokines such as TNF-α and IL-1β and IL-6 are involved at the different stages of plaque formation.[9] Red blood cell distribution width (RDW) has recently gained prominence as a prognostic biomarker in inflammatory conditions.[10] RDW is easily reported during complete blood cell count. Normal range of RDW is 11.5% to 14.5%. High levels of RDW have been shown to be associated with elevation of pro-inflammatory cytokines and unsuccessful reperfusion.[11] C- reactive protein (CRP) which is a well-studied inflammatory biomarker is produced by hepatocytes in response to IL-1β and IL-6.[12] CRP rises in AMI and peaks after reperfusion therapies.[13] Percutaneous coronary intervention (PCI) and thrombolytic therapy are effective to restore blood flow in patients with IHD, however, both of them are associated with rise in inflammatory responses.[14-16] The aim of the present study was to compare the effects of PCI and thrombolytic therapy on TLR4, cytokines, RDW and CRP in patients with acute MI.

MATERIALS AND METHODS

Study Subjects

The study was carried out in Shahid Madani Heart Hospital, Tabriz, Iran. 93 patients with ST elevation myocardial infarction (STEMI) were enrolled in the study. According to clinical conditions and cardiologist order, patients underwent either PCI or thrombolytic therapy. Patients were divided in two groups: PCI and thrombolytic. The exclusion criteria were as follows: previous Q-wave MI within 6 months (5 patients), autoimmune diseases (1 patients), inflammatory conditions (2 patients), advanced hepatic or renal disease and malignancy (2 patients) and receiving hydrocortisone during PCI (2 patients). Cardiovascular risk factors, medications, sex, age and previous medical history were recorded. White blood cell count, cholesterol, glucose, PT, PTT, BUN, creatinine, sodium and potassium were measured according to routine protocols. The ethical board of Tabriz University of Medical Sciences approved the study (No.57) and informed consent letter was obtained from all participants.

Blood Collection and Processing

A total of 10 ml blood was drawn from patients using a 21-gauge needle via antecubital venipuncture. Two ml was kept in EDTA anticoagulant tubes for flowcytometry and the rest for analysis of biomarkers. Blood collection was done in a time dependent manner: at the time of admission (0 h), 2 hours and 4 hours (2h, 4h) after reperfusion. Eight ml of blood was centrifuged immediately (3000×g for 5 min) to obtain serum. Serums were kept in–80^{o}C for future analysis.

PCI Protocol

Patients received clopidogrel (300 mg) and aspirin (300 mg) prior to PCI. Intravenous heparin (10,000 U) was administered before the procedure. Physicians decided the types of balloons and stents implanted during the surgery. All PCI procedures were carried out according to protocols of the hospital. After PCI, patients received clopidogrel (75 mg) and aspirin (75mg).

Thrombolytic Therapy

Patients in this group were given an IV dose of 1.5 million IU streptokinase over 1 hour at admission. Clopidogrel (300mg) and aspirin (300 mg) were also administered. Then both drugs were given as an oral dose of 75 mg. Patients also received beta-blockers, nitrates, enoxaparin or heparin, statins and opioids.

Measurement of TLR4 Expression on the Surface of Monocytes

Briefly, cells were stained for 30 minutes with monoclonal antibodies against human CD14 (Abcam,

UK) conjugated with fluorescein isothiocyanate (FITC), and TLR4 (Abcam, UK) conjugated with phycoerythrin (PE). Cells were analyzed by FACSCalibur flow cytometer and CellQuest software (BD Biosciences, US).

Measurement of TNF-α and IL-1β

A sandwich enzyme-linked immunosorbent assay (ELISA) was performed (Ray Bio, US). In short, 100 µl serum was added to microtiter plates. Thereafter, 100 µl prepared biotin antibody was added to each well and incubated for 1 hour at room temperature. Then 100 µl streptavidin solution was added and incubated for 45 minutes at room temperature. The intensity of the produced color was measured at 450 nm by Stat Fax 2600 (Awareness Technology, US) plate reader. For washing steps Stat Fax 2100 (Awareness Technology, US) was used.

Measurement of RDW and CRP

Levels of RDW were measured with the use of pocH-100i analyzer (Sysmex, Japan) as a part of blood cell count. Automatic chemistry analyzer (Toshiba, Tokyo, Japan) was used to detect CRP levels in sera of the patients.

Statistical Analysis

Data are reported as mean ± SD or numbers (percentage). Categorical variables were compared using one-way analysis of variance. Means were compared by wilcoxon signed rank test. *p* value less than 0.05 was considered to denote significant differences. All analyses were performed using SPSS 19.0 (IBM, USA).

RESULTS

Patients

A total of 83 STEMI patients (40 PCI treated, 43 thrombolytically treated) were recruited between Nov 2012 and May 2013. Baseline characteristics are presented in Table 1. In both groups, the mean age was 65 years. There was an excess of males; 62% in PCI group and 69% in thrombolysis group. There were no significant differences between the 2 groups regarding demographic, clinical, and biochemical parameters.

Table 1. Clinical and laboratory characteristics of study patients

Topics	PCI (n=40)	Thrombolysis (n=43)	*p* value
Age, years	65±9	65±7	0.7
Gender M/F	25 (62.5) /15 (37.5)	30 (69.7) /13 (30.2)	0.3
Diabetes mellitus	23 (57.5)	26 (60.4)	0.8
Hypertension	16 (40)	13 (30.2)	0.7
Hyperlipidemia	17 (42.5)	18 (41.8)	0.9
Smoking	11 (27.5)	14 (32.5)	0.6
Family history	7 (17.5)	11 (25.5)	0.4
Nitrates	27 (67.5)	28 (65.1)	0.3
Beta-blockers	31 (77.5)	31 (72)	0.6
Statins	24 (60)	21 (48.8)	0.7
ASA	33 (82.5)	34 (55.8)	0.6
Clopidogrel	19 (47.5)	16 (37.2)	0.5
ACE inhibitors	26 (65)	30 (69.7)	0.3
ARBs	12 (30)	13 (30.2)	0.6
CCBs	12 (30)	8 (18.6)	0.6
WBC count, U/L	7.8±0.2	7.1±0.4	0.4
Glucose, mg/dl	114±23	99±21	0.3
Total cholesterol, mg/dl	193±40	188±35	0.7
Triglyceride, mg/dl	119±53	110±62	0.6
LDL, mg/dl	141±31	140±21	0.8
HDL, mg/dl	43±6	40±9	0.5

Data are shown in mean±SD and number (%).

ACE: angiotensin converting enzyme, ASA: acetyl salicylic acid, ARB: angiotensin receptor blockers, CCB: calcium channel blockres.

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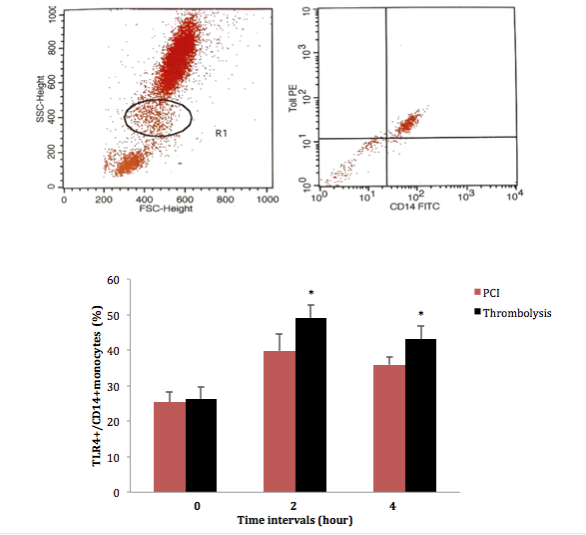

Figure 1. Representative dot plot showing TLR4$^+$/CD14$^+$ monocytes. Ten thousand cells were analyzed by flowcytometry. Data are shown as mean±SD. Ten thousand cells were analyzed by flowcytometry in PCI group and thrombolysis group; baseline (0h), two hours (2h) and four hours (4h) after treatment, $^*p$<0.01 vs. PCI.

Monocyte Expression of TLR4

At baseline, monocyte expression of TLR4 was 25.3±2.9 % in PCI group and 26.3±3.2% in thrombolysis group. In PCI group, TLR4$^+$/CD14$^+$ monocytes were 39.8±4.7% and 35.9±2.3%, two and four hours after PCI, respectively. Monocyte expression of TLR4 was significantly high in thrombolysis group as compared to PCI groups, two and four hours after beginning the treatment (49.1±3.6% and 43.1±3.7%, respectively, p<0.01; Figure 1).

Levels of TNF-α, IL-1β, RDW and CRP

In PCI group, serum level of TNF-α was 14.4±2.1 pg/ml prior to the intervention. It reached to peak levels of 21.5±2.3 (pg/ml) and 22.4±3.1 (pg/ml) two and four hours post PCI. In the thrombolysis group, serum level of TNF-α was higher as compared to PCI group at the same intervals (25.1±2.6 pg/ml and 23.1±2.9 pg/ml, respectively, Table 2).Two and four hours after PCI, an increase was seen in the serum concentrations of IL-1β regarding to the baseline value (11.1±2.4 pg/ml, 15.5±2.4 pg/ml, 15.2±2.8 pg/ml,). Compared with the PCI patients, the serum levels of IL-1β did not show any major change in thrombolysis group at 0 hour, 2 hours and 4 hours after receiving streptokinase (10.3±3.4 pg/ml, 14.1±2.3 pg/ml and16.7±3.4 pg/ml respectively, Table 2). Differences between groups failed to reach a statistical significance. At base line, mean RDW was 12.2±1.3% in PCI group and 11.9±2.4% in thrombolysis group. After treatment, there was no significant change in the levels of RDW neither in PCI nor in the thrombolysis group (Table 2). There was no significant difference in levels of CRP in two groups (1.1±0.1 vs 1.2±0.2 mg/dl) at baseline. After reperfusion therapies, CRP levels increased in both groups at both intervals. The difference after 4 hours was statistically significant (p<0.01, Table 2).

Table 2. Levels of TNF-α, IL-1β (pg/ml), RDW (%) and CRP (mg/dl) at different interval times (hour)

Group	TNF-α			IL-1β			RDW			CRP		
	0	2	4	0	2	4	0	2	4	0	2	4
PCI (n=40)	14.4±2.1	21.5±2.3	22.4±3.1	11.1±2.4	15.5±2.4	15.2±2.8	12.2±1.1	12.7±2.1	12.4±1.4	1.1±0.1	1.3±0.5	1.3±0.4
Thrombolysis (n=40)	13.2±3.7	25.1±2.6*	23.1±2.9	10.3±3.4	14.1±2.3	16.7±3.4	12.1±2.4	12.5±1.5	12.4±1.9	1.2±0.2	1.4±0.3	1.9±0.3$^\#$

$^*p$<0.05 vs. PCI, $^\#p$<0.01 vs. PCI

DISCUSSION

The key finding of the present study was that compared with PCI, thrombolytic therapy was associated with significant increase in monocyte expression of TLR4 and cytokine levels in patients with STEMI. In addition, it raised CRP levels. During reperfusion several important substances are released. Heat shock proteins (HSP) which are frequently seen both in atherosclerotic plaques and blood are well documented ligands for TLR4 and can propagate inflammatory responses. In addition to inflammation, it is also very important to consider cellular response to oxidant stress. Oxidants activate a signal transduction cascade that may engage the cell-death pathway and provoke apoptosis. These factors can contribute to development of reperfusion injury.[17] In the present study, we showed that both reperfusion therapies augment monocyte expression of TLR4 and cause an increase in production of inflammatory cytokines. Evidence from several lines of investigations has shown that both PCI and medical reperfusion therapy are able to upregulate the inflammatory mediators and cause myocardial injuries.[15-17] However, these inflammatory damages outweigh the detrimental effects of extensive occlusion of the coronary arteries which is a dangerous gate to the myocardial necrosis. Versteeg et al, showed that monocyte TLR4 expression and serum levels of TNF-α decreased after PCI in patients with stable angina. They suggested paradoxical effects of oxLDL and nicotinic anti-inflammatory pathway of the vague nerve as the two possible mechanisms causing this attenuation, however, they reported a modest increase in intracellular level of TNF-α after PCI procedure.[18] It is not known with certainty that how PCI is associated with lower inflammatory activity compared to thrombolysis. Rapid thrombolysis which occurs in response to thrombolytic and antithrombotic therapies may lead to larger inflammatory responses. In this study, we demonstrated that serum levels of cytokines increased after reperfusion therapies. It is proposed that gradual infiltration of $TLR4^{+}$ monocytes in developing plaques and production of cytokines in concert with other important players can deteriorate atherosclerosis.[6,7,19]Cytokines impairs vasodilatation, , reduce coronary flow and thus contribute to facilitate ischemic damages. Vink et al. revealed that stimulation of TLR4 on adventitial fibroblasts augmentes neointima formation, an effect that is reduced in TLR4-defective mice.[20] It should be noted that monocytes are not sole players of inflammation, hence, endothelial cells and lymphocytes are capable of producing inflammatory cytokines.[21,22] Ridker's work proved that patients with higher levels of TNF-α are more prone to recurrent coronary events after MI, suggesting the role of cytokines in pathogenesis of MI.[23] On the other hand, IL-1β induces activation and proliferation of monocytes and stimulates further production of cytokines in atheroegensis. A study by Aggarwal et al. indicated that in patients undergoing coronary stenting, an increase in IL-6 can be detected 1 hour after PCI, and thus IL-6 may be an early initiator of the systemic inflammatory response to stenting.[24] In our study, CRP had a significant rise after reperfusion therapies. It is documented that maximum rise of CRP is generally observed 48 hours after stent implantation. [13] Walter et al. found that tertiles of CRP levels were independently associated with a higher risk of major adverse cardiac events (MACE) and angiographic restenosis after stenting.[25] Moreover, Buffon's investigation demonstrated that baseline CRP was independent predictor of restenosis.[26] Of note, recent guideline published by European Society of Cardiology (ESC), has enumerated several weak points for CRP to be used for risk assessment in CAD patients, including multiplicity of confounders, lack of precision, lack of specificity and lack of therapeutic strategies.[27] Despite these facts, CRP is still important in research and clinic. Fresh clinical findings, have addressed red blood cell distribution width (RDW), as a strong and independent predictor of adverse outcomes in patients with cardiovascular disease and heart failure.[28] Baysal's study showed that the prevalence of failed thrombolysis was significantly higher in patients with an RDW more than 14.3% than in those with an RDW of 14.3%.[11] Although the precise mechanism involved in the increased RDW is still unclear, there is an evidence for a relationship between systemic inflammation and oxidative stress. Inflammatory cytokines may change red blood cell maturation by disturbing the red cell membrane, leading to increased RDW, and free radicals directly damage erythrocytes and lead to shortened erythrocyte survival, resulting in elevated RDW.[28,29] Evidence from diverse sources has provided a wealth of data suggesting that inhibition of TLR4 or TLR4 related downstream activity can subsequently induce injuries related to TLR4.[30,31] In our previous studies , hydrocortisone and everolimus were able to

reduce monocyte expression of TLR4 in patients underwent PCI.[32,33] At present, there are two hurdles against achievement of inflammation inhibition in MI setting; firstly, evidence points to the contribution of TLR4 in angiogenic processes which are very important in collateral perfusion.[34] Secondly, there is lack of evidence proving the effectiveness of immunosuppressive therapies.[35,36]

In conclusion, this study shows that thrombolytic therapy is associated with greater inflammatory response compared to PCI. However, both reperfusion therapies are linked with inflammatory responses. It seems that more basic and clinical research should be done regarding the role of TLR4 and inflammation in atherosclerosis. The fire inside the plaques should be silenced, yet it is not known when and how to do it. There are several limitations to our study. First, a relatively small number of patients were studied, so our findings may be confirmed in larger studies. Second, inflammatory responses were evaluated over 4 hours after reperfusion therapies. Therefore, it is possible that dramatic changes occur over the next hours. Third, C-reactive protein might reflect a periprocedural reaction which is not always associated with reperfusion therapies.

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