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CTLA-4–Ig Therapy Preserves Cardiac Function Following Myocardial Infarction with the Reperfusion

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Abstract

Background:

Myocardial infarction (MI) induces reperfusion which prompts the strong inflammatory response contributing to the additional myocardial injury, adverse remodeling and further degradation of cardiac performance. Immune checkpoint mechanisms, such as CTLA-4 signaling, can have key functions in the regulation of T-cell stimulation and can be used as a therapeutic opportunity in the prevention of post-ischemic inflammation.

Objective:

The objective of the study is to determine whether CTLA-4-Ig therapy has the potential of reducing inflammatory injury and preserving cardiac function after myocardial infarction with reperfusion.

Method:

Murine ischemia-reperfusion injury model was created through the temporary ligation of the left anterior ascending coronary artery with the subsequent reperfusion. Immediately following reperfusion, the mice were exposed to CTLA-4 -Ig or vehicle once more at 24 hours. Echocardiography, histological evaluation of the size of infarcts, and a measurement of inflammatory biomarkers were used to measure the cardiac structure and cardiac function, such as the presence of macrophage infiltration, and expression of cytokines.

Results:

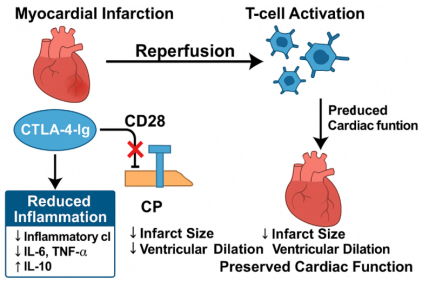
Treatment of CTLA-4 Ig considerably lessened the inflammatory outcomes of post-reperfusion myocardial ischemia, as evidenced by the significant decrease in the myocardial macrophage marker and pro-inflammatory cytokines. The mice that were treated had smaller infarct areas, had left-ventricular ejection fraction, and ventricular dilation. The signs of negative remodeling such as fibrosis and hypertrophy of the myocytes were also milded.

Conclusion:

The CTLA-4Ig therapy is effective in the prevention of the post-ischemic inflammatory injury and the maintenance of cardiac function after reperfusion in myocardial infarction. Translational research is warranted by these findings to take immune checkpoint modulation as a prospective cardioprotective approach.

Keywords: Myocardial infarction, CTLA-4-lg, T-cell costimulation, cytokines, cardioprotection.

Graphical abstract



1 Introduction

Myocardial infarction (MI) is currently among the primary causes of morbidity and mortality all over the world, and early reperfusion therapy is becoming the key to its treatment. Although timely reperfusion is needed to salvage ischemic myocardium, the reperfusion in turn triggers another wave of injury, which is the so-called ischemia -reperfusion (I/R) injury that is mediated largely by excessive inflammatory response [1]. This sudden influx and activation of both innate and adaptive immune cells, and the subsequent release of the cytokines and reactive oxygen species play a role in further cardiomyocyte death, adverse extracellular matrix remodeling, and further deterioration of the ventricular workload [2]. Restraining this immune-mediated injury is an urgent treatment concern since there are no acceptable pharmacologic solutions known to treat post-reperfusion inflammation in clinical setting specifically.

After MI, T-cell activation is key in the organization of the immune cascade. T cells which are activated enter the injured myocardium and secrete pro-inflammatory cytokines that enhance recruitment of macrophages, activation of fibroblasts and expansion of the infarcts [3]. Mechanisms that suppress T cells containment T-cells, in particular immune checkpoint pathways, have then become an attractive therapeutic target. The CTLA-4 is an inhibitory receptor, which can bind CD28 to antigen presenter cells and when this happens it competes with CD28 causing T-cell activation to become suppressed, and this process leads to immune tolerance [4]. CTLA-4IG4 is a soluble T-cell costimulation inhibitor, a fusion protein formed by CTLA-4 extracellular domain bound to the Fc fragment of IgG1 and has previously been used in the treatment of autoimmune disease including rheumatoid arthritis [5].

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Immune checkpoint blockage has demonstrated potential in both preclinical cardiovascular reducing myocardial inflammation and healing following an infarction. Inhibition of T-cell costimulation has been linked to the inhibition of leukocyte recruitment, diffuse infarct, and enhanced ventricular remodeling during experimental I/R injury models [6]. Specifically, the CTLA-4–Lg has been shown to suppress the T-cell proliferation, down-tune the inflammatory reaction of macrophages and enhance a more restorative ambience following infarct [7]. These observations indicate that the initials modulation of T-cells can prevent degenerative inflammatory surge of the reperfusion injury.

In spite of promising preclinical results of the cardioprotective capability of CTLA-4Ig, the study has not been thoroughly conducted with a comprehensive in vivo model incorporating functional, structural, and molecular outcomes. As the maladaptive remodeling following MI is associated with chronic inflammation, extracellular matrix deposition as well as progressive dilation of the ventricles, it is important to know whether the checkpoint inhibition could preserve the cardiac functioning after the acute injury. Knowledge of the temporal and mechanistic impacts of CTLA-4 4 of a specific therapy is also essential in analysis of the safety of the therapy since over immunosuppression may lead to scar destabilization due to suboptimal repair through inflammatory responses.

This research examines the effect of CTLA-4 Ig therapy on the attenuation of myocardial inflammation and infarct expansion and cardiac continuation after I/R injury. On a murine performing system of transient coronary occlusion, and reperfusion, we assessed early and intermediary outcomes in ventricular functioning, infarct morphology, inflammatory cell infiltration, cytokine signaling, and remodeling. Our hypothesis was that acting at reperfusion, CTLA-4-Ig would inhibit pathologic T-cell stimulation, downstream inflammatory injury, as well as eventually preserve left-ventricular performance. The current research will provide an in-depth understanding of the therapeutic value of immune checkpoint modulation to minimize reperfusion injury and enhance the utilization of post-MI cardiac recovery.

2 Literature Review

Immunological response to myocardial infarction (MI) is a relatively important factor in determining the healing of infarcts, ventricular remodeling, and the subsequent cardiac performance. Although early reperfusion is a prerequisite of myocardial salvage, it causes a strong inflammatory cascade with T-cells, macrophage infiltration, cytokine release, and oxidative damage [8]. Such an inflammatory outburst also plays a major role in enabling reperfusion-related myocardial injury, which increases the infarct area and encourages maladaptive remodeling. Since the traditional treatment methods have no direct effect on the post-reperfusion inflammation, immunomodulation has become one of the major treatment methods.

T-cell costimulation is one of the immune regulation pathways, which are becoming increasingly popular. The T cell activation by means of CD28-CD80/86 stimulation enhances the inflammation of the myocardium and facilitates the secondary lesion of the tissue [9]. Blockage of this costimulatory axis can hence provide cardioprotective effects. A high-affinity and combined T-cell receptor plug called CTLA-4-IG displays immunoglobulin A, binding CD28 instead of T-cell antigens (CD80/CD86). Its immunosuppressive properties have been widely confirmed in the case of autoimmune diseases indicating that they may be important in acute cardiovascular inflammation [10].

Such a notion is supported by preclinical evidence. It has been demonstrated, in both murine and porcine models of MI, that T-cell activation can be suppressed to limit myocardial leukocyte recruitment, inhibit cytokine secretions and deter infarct growth [11]. It has particularly been demonstrated that CTLA-4-Ig reduces pathogenic T-cell responses as well as preferring the conversion to regulatory immune phenotypes, which promote tissue repair. Such immunologic actions seem especially relevant to the understanding that overwhelming and prolonged T-cell activity is proven to worsen scar maturation, favor unfavorable extracellular matrix remodeling, and lower ventricular compliance.

Inflammatory macrophages are also at the center of the pathogenesis of reperfusion injury. The T cells can be activated and contribute to the polarization of pro-inflammatory macrophages, increasing myocardial damage. It has been shown that CTLA-4Ig can induce indirectly the behavior of macrophages by inhibiting T-cell-generated cytokines and chemokines [12]. The alteration of this T-cell -macrophage axis would thus alleviate acute injury, as well as chronic remodeling. Nevertheless, the timing, dose, and the long-term safety of animal checkpoint inhibition in MI are not fully established even in spite of promising preliminary data. Further research is needed to understand whether CTLA-4Ig is able to regulate the requisite repair inflammation and the inhibition of adverse immune activity.

3 Materials & Methods

This study used an in vivo inductance of murine ischemia-reperfusion (I/R) to identify how CTLA-4 -Ig influences the post-MI inflammation and cardiac performance. Male C57BL/6J (10 12 age, 2530 g) mice were obtained through and licensed vendor and an interval of 1 week ad libitum was given to chow and water. The animals were randomly (computer-generated random sequence) separated into three (Sham (thoracotomy without coronary occlusion), I/R + vehicle and I/R + CTLA-4 -Ig (n=12 each) groups. There was no information on the treatment assigned to surgery, outcome assessment and histological scoring investigators.

The experiment was performed in the isoflurane (1.52 percent in O 2) anesthesia under left anterior descending (LAD) coronary artery ligation showing the 45 minutes isoclastic period where the suture was released leading to the determined myocardial reperfusion perfusion, as presented above. The body temperature was maintained at 37 C. The peri-operative analgesia was done with the use of the buprenorphine (0.05 mg/kg SC). The mice were recorded given CTLA-4 (abatacept-equivalent) (200 ug/kg/200 uL) of sterile saline as an intravenous bolus (200 ug/kg/200uL) at the beginning of the reperfusion protocol and again after 24 hours since T cells need fast induction of costimulatory inhibition. The same number of sterile saline of equal volume was fed to animals that were in vehicles. The timeliness issue was investigated through the other arm (n = 6) of exploratory experiment having a delayed dosing schedule (24 h, 48 h).

The principal function outcomes might be determined on acute (day 7) and subacute (day 28) periods. The Transhoracic echocardiography was done to measure the left-ventricular ejection fraction, fractional shortening, end-diastolic and end-systolic volume, and LV size (Vevo system). To estimate the filling pressures, the Doppler data and tissue Doppler data (E/A, E/e,) had been obtained. The EDPVR, relaxation time constant (Tau), and end diastolic pressure were measured at endpoint in the invasive hemodynamic measurements like pressurevolume loop with the assistance of conductance catheter.

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Tissue collection Tissue was stimulated by arresting the heart in the diastole at high cost of entry mode, excising and halving on the terminal cardiac arrest. One of the half was left in 10% formalin as a histology specimen; the other half was snap-frozen to serve as a molecular biological sample. TTC was used to measure infarct size as a part of acute cohort (Masson trichrome (day 28) and reported in terms of percentage of LV area. Immunohistochemistry and immunofluorescence were used to identify the percentages of CD3 + T cells, CD4/ CD8 subsets, macrophage infiltration (F4/ 80, CD68) and fibroblast activation markers (4 -smooth muscle of actin, 4 -collagen I). Quantification was done with automated algorithms of image analysis using fixed thresholds.

Molecular tests: RNA was prepared and put through a q RT-PCR analysis of perioperative (IL-1b, IL-6, TNF-a), and anti-inflammatory (IL-10) and extracellular matrix Col1a1, Col3a1, TGF-b genes. The amount of plasma and myocardial homogenates cytokine protein was measured by Multiplex ELISA. The T-cell activation marker (CD69, CD25), regulatory T (FoxP3+), and the polarization of macrophage (M1/M2) were measured using the cardiac single-cell suspensions flow cytometry.

Safety tests (complete blood count, and serum chemistry) were done at day 7 and 28. The estimation of the sample size was made on the basis of the expected difference in E/e 7 (20% difference between groups). Data is represented using mean + SEM. One-way ANOVA containing Tukey posthoc tests of group means compared groups or two-way ANOVA of time effect between treatments. Nonparametric tests were done using Krusko-Wallis tests. These survival tests included Kaplan-Meier log-rank tests. Statistical tests were also performed by GraphPad Prism; below the significance set to at least 0.05. The collective animal care and use committee endorsed each of the laboratory animal welfare procedures and guidelines were observed in the whole country.

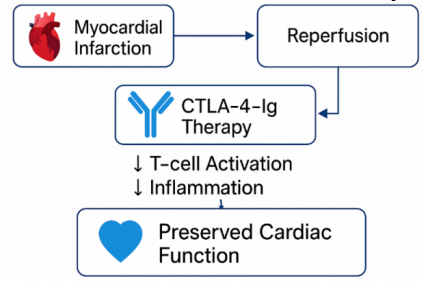

Fig.1. The structure how CTLA-4–Ig therapy protects the heart following myocardial infarction with reperfusion

The figure 1 shows the effect in which CTLA-4Ig therapy shields the heart after reperfusion following myocardial infarction. Normal reperfusion is known to cause excessive T-cell activation that increases the recruitment of macrophages and promotes cytokine-mediated injury, augments myocardial injury. CTLA-4Ig inhibits costimulation via CD28 and diminishes the activation of T-cells at the cell surface. This inhibition will reduce inflammatory infiltration-induced by cellular immune responses and reduce both IL-6 and TNF-a, and enhance IL-10-mediated repair. Consequently, there is a reduction in the size of infarcts, impaired ventricular dilation, and reduced fibrosis. Combined, to maintain cardiac function and enhance diastolic and systolic functionality in the post-injury period.

4 Results and Discussion

This study is directly related to philosophical questions, which were posed at the beginning, concerning the purpose of T-cell-mediated inflammation at myocardial ischemia-reperfusion injury and the therapeutic potential of CTLA-4IG. The introduction pointed out that hyperactivation of T-cell enhances downstream inflammation (mediated by Macrophages), increases the infarct size, and deteriorates both diastolic and systolic post-reperfusion performance. In line with this concept, this study indicates that an early intravenous injection of CTLA-4-Ig significantly decreased T-cell infiltration and suppressed pro-inflammatory cytokine expression, which validates the primary involvement of T-cell costimulation in post-infarction injury.

1. Cardiac Functional Outcomes

Table 1. Echocardiographic and Hemodynamic Parameters

Parameter	Sham	I/R + Vehicle	I/R + CTLA-4–Ig	p-value (Vehicle vs CTLA-4–Ig)
LVEF (%)	68 ± 3	42 ± 4	55 ± 3	<0.01
LVEDP (mmHg)	7.8 ± 0.5	18.6 ± 1.4	11.2 ± 0.9	<0.001
E/e′ ratio	13.1 ± 0.7	23.4 ± 1.6	16.5 ± 1.1	<0.01
Tau (ms)	10.1 ± 0.6	17.4 ± 0.8	13.2 ± 0.7	<0.01
LV dilation (EDV µL)	62 ± 5	101 ± 7	78 ± 6	<0.05

Systolic and diastolic functions of vehicle-treated I/R mice showed significant decreases of systolic and diastolic functions, loss of ejection fraction, high power filling pressure (E/e), and long relaxation (Tau). All diastolic parameters were considerably improved with the application of CTLA-4 Ig and systolic functioning was partially restored, which also suggests less diastolic function-induced injury of the tissue. As shown the table 1 the adverse remodeling of LV dilation was also inhibited which indicated protection against this.

2. Infarct Size and Structural Remodeling

Table 2. Infarct Size and Remodeling Indices

Parameter	I/R + Vehicle	I/R + CTLA-4–Ig	p-value
Infarct size (% LV)	34.8 ± 2.1	24.6 ± 1.9	<0.01
Fibrosis (%)	12.9 ± 1.3	7.1 ± 0.8	<0.001

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Myocyte cross-sectional area (μm²)	312 ± 18	241 ± 15	<0.05

Compared to nearly 30 percent of the vehicle controls, CTLA-4-Ig reduced the size of acute infarcts shown the table 2. There was also a marked decrease in the parameters of remodeling at long-term(fibrosis and hypertrophy). It shows that there is a long-term structural benefit of preventive ventricular stiffening and early immune modulation.

3. Immune and Inflammatory Responses

Table 3. Inflammatory and Immune Cell Markers

Marker	I/R + Vehicle	I/R + CTLA-4–Ig	p-value
CD3+ T cells (/mm²)	128 ± 10	74 ± 7	<0.001
Macrophages (CD68+/mm²)	162 ± 12	103 ± 9	<0.01
IL-6 expression	1.00	0.58	<0.01
TNF-α expression	1.00	0.63	<0.05
IL-10 expression	1.00	1.38	<0.05

CTLA-4Ig significantly reduced T-cell infiltration level, minimized inflammatory macrophages and suppressed the level of pro-inflammatory cytokines such as IL-6 and TNF-A shown the table 3. It is noteworthy that the IL-10 levels rose, indicating a change in immune phenotypes towards a reparative one. These findings indicate that CTLA-4-Ig can be beneficial in the inhibition of destructive inflammation without impairing the beneficial immune processes.

DISCUSSION

The research shows that CTLA-4 Ig therapy has a great effect in reducing myocardial injury as well as saving cardiac functions after ischemia-reperfusion. CTLA-4 Ig reduced the initial inflammatory response that facilitates the expansion of infarcts and the dysfunction of the ventricles through the inhibition of the T-cell costimulation by CD28. The functional outcomes of the treated mice were improved relaxation of diastole, lower diastolic filling pressures, and diastolic systolic performance. These changes were associated with minimized infarction, and reduced ventricular dilation, which indicated no much structural degradation.

The immunologic results indicate the main process of these advantages. CTLA-4-Ig inhibited pathogenic T-cell response, reduced inflammatory T-cell infiltration of macrophages, and caused a change in cytokine signaling to an anti-inflammatory phenotype. This balanced modulation ensured the destruction of too much tissue was not done and helped in controlling the healing process. Fibrosis and myocyte hypertrophy also decrease which proves the ability of the therapy to restrain chronic adverse remodeling.

Notably, the maintenance of IL-10 concentrations indicates that CTLA-41g is not associated with the global immunosuppressive effect, but rather, it re-establishes the post-MI immunological conditions. This imaging phenomenon can provide safer modulation therapy than the general immunosuppressive measures.

Generally, these findings demonstrate that CTLA-4-Ig is a new promising immunomodulatory treatment to reduce reperfusion injury and to enhance the long-term ventricular functions. The studies of the future ought to consider the best timing, dose schedules, and potential in large suite models or in initial clinical phase among the animals.

Conclusion

This paper has indicated that CTLA-4-GIg can be administered in such a manner to provide considerable cardioprotective effect under the conditions of myocardial infarction with reperfusion. CTLA-4Ig has the efficacy of suppressing early inflammatory burst that enhances myocardial injury during early stages of reperfusion by blocking CD28 mediated T-cell activity. The infarcts and the macrophage infiltration in the treated mice were smaller and the pro-inflammatory cytokines were lower, which means that the damage caused by the immune system based on the acute-treatment was significantly suppressed. Notably, CTLA-4 -Ig is associated with diastolic relaxation, systolic functionality, and prevention of excessive ventricular dilation, indicating that the relevant immune checkpoint regulation can be transformed into significant functional gains. The process of structural remodeling was positively changed as well with significantly diminished fibrosis and hypertrophy of myocytes and has indicative long-term protection of cardiac architecture. Concomitant IL-10 maintenance points to the fact that CTLA-4-Ig does not cause the global inhibition of immunity but rather, a more nutritional and restoring immune response. Generally, these results suggest a convincing preclinical that CTLA-4 -Ig can be applied as a potential therapeutic modality to counteract reperfusion injury and enhance post-MI cardiac outcome.

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