Treatment Trends of Myocarditis with Coxsackievirus B3 infection

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Abstract

Viral myocarditis occurred after viral invasion of the cardiocytes and followed by the releasing of viral particles and inflammatory cells. Acute viral myocarditis is relatively common phase of the disease cured spontaneously in some cases or leading to sever acute heart failure and cardiac damage ended with chronic heart failure with increased mortality rate. The fully understand mechanism of viral myocarditis is unclear but some hypothesis trying to illustrate the events of the disease.

Regardless of etiological cause, myocarditis is treated depending on disease phase and by following the instructions of Heart Failure Society of America guideline.

This article provides the basic knowledge available in literatures to review some important informations of viral myocarditis, with a special focusing on viral myocarditis caused by coxsackievirus B3 infection.

Key words: Viral Myocarditis, Coxsackievirus B3, Ribosomal nucleic acid, Antiviral drug.

الاتجاهات في علاج التهاب عضلة القلب الناتج عن الاصابة بفيروس كوكساكي B3

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الخلاصة:

التهاب عضلة القلب الفيروسي يحدث نتيجة الاصابة الفيروسية ودخول الفيروس لخلايا عضلة القلب والذي يعقب بإطلاق الجسيمات الفيروسية والخلايا الالتهابية. يعد التهاب عضلة القلب الفيروسي الحاد مرحلة شائعة نسبيًا من المرض الذي يتم علاجه تلقائيًا في بعض الحالات أو يؤدي إلى قصور حاد في القلب وتلف في القلب ينتهي بفشل القلب المزمن مع زيادة معدل الوفيات. آلية الفهم الكامل لالتهاب عضلة القلب الفيروسي غير واضحة ولكن هناك بعض الفرضيات التي تحاول توضيح أحداث المرض.

بغض النظر عن العامل المسبب للمرض ، يتم علاج التهاب عضلة القلب اعتمادًا على مرحلة المرض واتباع إرشادات جمعية فشل القلب الأمريكية.

توفر هذه المقالة المعرفة الأساسية المتوفرة في الأدبيات المنشورة لمراجعة بعض المعلومات المهمة عن التهاب عضلة القلب الفيروسي، مع التركيز بشكل خاص على التهاب عضلة القلب الفيروسي الناجم عن العدوى بفيروس كوكساكي ب.

الكلمات المفتاحية: التهاب عضلة القلب الفيروسي، فايروس كوكساكي ب،الحامض النووي الرايبي، الدواء المضاد للفيروسات.

Introduction

Viruses are causing diseases accompanied with clinical symptoms such as flu, sore throat, gastritis and others which recovered after treatment or resolved spontaneously. Sometimes after viral infection many complications may develop, one of these complications is myocarditis, an inflammation of the heart muscle [1].

Corvisart used the term "myocarditis" in the 19th century for the first time, but this term was ended after the recognition of coronary artery disease as the main cause for heart disease [2].

Myocarditis (inflammatory cardio-myopathy) is an inflammation of the heart and probably leading to dilated cardiomyopathy (DCM), it is characterized by clinical or histopathological features, with characterized broad clinical spectrum ranging from asymptomatic to the onset of heart failure ending with cardiac death and this clinical picture is vary from person to person. Many informative data and studies tried to focus on the relation between infectious agents (bacteria, viruses, and fungi) or autoimmune reaction that leads to myocarditis [3].

Regarding the etiological and causative agents, the most common viruses causing myocarditis are enteroviruses (especially coxsackievirus B), adenovirus, parvovirus B19, hepatitis virus, and herpesviruses especially human herpesvirus 6, Epestien Barr virus and human cytomegalovirus [4-6].

Viral myocarditis is a main cause of an acute and chronic dilated cardiomyopathy, many studies especially seroepidemiological and molecular studies stated that since 1950s to 1990s coxsackievirus B was linked to the outbreaks of myocarditis [6,7].

Pathological changes in myocarditis is one of the heart muscle injury which caused by necrosis after infectious agent invasion and then causing triggering innate immune response at the site of injury including interferon gamma, natural killer cells, and nitric oxide [8].

Myocarditis is divided into three distinct phases, destruction of cardiomyocytes due to the lysis that caused by the virus is occurred in the first stage which leading to increase entrance of the virus and cardiac dilatation. Released particles from the invasive virus and cardiac proteins are phagocytized by antigen presenting cells that transported to the regional lymph nodes. Most patients recovered at this stage but in others when viral myocarditis suspected to be a primitive cause of autoimmune disease affecting the heart muscle, the disease will continue. The standard method for diagnosis was mainly based on Dallas criteria which depends on histopathological changes in the endomyocardial biopsy. However, the Dallas criteria now is not sensitive for the diagnosis of myocarditis because this criterion doesn't match with viral genome in the biopsy and also it is considered as an invasive method [9,10].

Regarding the hypothesis which believed that the most common viruses have a distinct role in autoimmune myocarditis such as enteroviruses (especially coxsackievirus), herpes viruses (especially Epstein Barr virus, human herpesvirus 6, and cytomegalovirus) and hepatitis viruses, a previous study on the inflammatory reactions that produced after infection by DNA and RNA viruses suggested that the viruses have non-specific role in myocarditis [3]. While other studies concentrated on the viruses that induced myocarditis may lead to autoimmune disease, and a study done by Andreoletti *et al.*, registered that up to 40% of patients who died with acute myocardial infection had active coxsackievirus B infection revealed cytoskeletal disruption in cardiomyocytes [11].

Pathogenesis and clinical presentation of viral myocarditis

The pathogenesis of viral myocarditis was studied previously through numerous animal model researches, all those studies showed that the infective virus enters via

receptors to the cardiac muscle and macrophages, initiating a cytotoxic effect [12,13]. The events of pathogenesis are not illustrated very well, but a serological study on enteroviruses registered the association between the elevated viral titer and the clinical feature of acute heart failure. Other studies proved that both immune response and direct viral destructive effect on myocardial tissue are explain the pathogenesis of viral heart disease [14,15]. The first immunological cells activated when the virus enter the heart muscle is natural killer (NK) cells, the role of NK cells is restricted to viral replication with production of interleukin (IL)-1β, tumour necrosis factor α (TNFα), γ interferon, and IL-2β, all these cytokines have contributed in beneficial and harmful effect by reducing the effects of viral infection and improve the heart function of murine model or by causing heart disruption [16,17].

The second inflammatory cells enrolled in the myocardial tissue of murine model infected with virus are T lymphocytes which have a role in viral clearance and immune mediated cardiac damage via the destruction of infected cells of the heart tissue. *In-vitro* study has been suggested that T lymphocytes may lead to cardiocytes injury due to molecular mimicry and similarity between viral protein and myocardial antigen that directed T cells to destruct myocardial tissues instead of eradicate the virus and the damage of the cardiac cells itself will stimulate more cell lysis [18].

A clinical trial showed that the prescription of immunosuppressive therapy didn't give good response in reducing of viral damage, while in other clinical study there was an improvement in patients with myocarditis after consumption of immune globulin [19,20].

Viral myocarditis categorized into acute and chronic phase. Viremia will occur after viral infection and entrance to the cardiocytes, the acute phase is accompanied with myocytes death and stimulation of immediate (innate) immune response through interferon gamma, NK cells, and nitric oxide [21]. The released viral particles and host protein of cardiac tissue carried by antigen-presenting cells outside the heart reaching the lymph nodes. Most patients with acute viral myocarditis have been recovered but many patients may enter the chronic phase of the disease which accompanied with adaptive immune response when the antibody against virus and against cardiac proteins are produced parallel with the proliferation of effector T cells. When the third phase starts, the immune reaction will reduce and down regulation will occurred then the fibrosis replaces the immune cells infiltration into the site of affected tissue in myocardium [22].

The clinical symptoms of viral myocarditis are varies ranging from ischemic like-chest pain to syncope due to acute heart failure and life-threatening heart failure [23]. Some patients with acute viral myocarditis are suffering from flu-like syndrome because of respiratory or gastrointestinal infections [24]. There is an enlargement in ventricular chambers, ventricular arrhythmia and irregularity in ST-T segments changes with elevation of cardiac enzymes (Creatine kinase/CKMB or protein troponin I). Angiography is very essential to distinguish between acute viral myocarditis and acute coronary syndrome. The acute phase of viral myocarditis is lasting from 2-4 weeks and the patients presented with non-specific symptoms such as prolonged angina, dyspnea, fatigue, and impaired ventricular function [25,26].

Acute viral myocarditis progressed to chronic viral myocarditis if the immune response failed to eliminate the causative agent. Viral myocarditis refers to the chronic phase when the acute phase does not disappear within 2-4 weeks or after recurrent symptoms appear [27,28].

Treatment

Many patients with myocarditis are suffered from mild symptoms and usually

self-limiting will occur, so first treatment for these cases is concentrating on supportive therapy, including supplemental oxygen, rest, and antibiotics for secondary bacterial infection in affected myocardial tissue [4-6].

The treatment of the viral myocarditis has been prescribed to the patient after the diagnosis of viral myocarditis which depends on the physical examination, estimation the elevated level of cardiac biomarkers such as, troponin I or troponin T, echocardiography, and cardiac Magnetic resonance imaging (MRI) [28].

The limitation of physical activities is highly recommended for all patients of myocarditis in general, this limitation is at least for six months or until complete healing and disappearing of clinical symptoms confirmed by MRI or cardiac biopsies. After complete the consumption of prescribed therapy for the patients with acute viral myocarditis, the patients should prevent from aerobic exercises for several months and could be return to this type of exercise when severity of the disease is reduced [29].

In general, the required therapy of viral myocarditis varies depending on the disease phase and should follow the the American College of Cardiology (ACC), the American Heart Association (AHA), and the European Society of Cardiology (ESC) and Heart Failure Society of America guidelines [30]. In acute viral myocarditis, symptomatic heart failure and DCM patient especially with hemodynamic stability may get benefit when uses angiotensin converting enzyme inhibitors or angiotensin receptor blockers. The treatment by β-adrenergic blockers may improve the function of left ventricular, relieve the symptoms of heart failure, and reduce inflammation [31,32].

The acute phase of viral myocarditis occurred during few days or weeks, so the antiviral agents have low effect on the disease. In addition to that the low sensitivity of myocardial biopsy in the diagnosis of viral infection make the antiviral chemotherapy has no value in the treatment; however; a good response occurred in mice with acute myocarditis when administered Ribavarin and interferon alpha at the time of virus inoculation [33,34].

Several studies and experiments suggested that acute and some cases of chronic myocarditis happened due to an immune reaction of T cells and autoimmune antibodies. However, data from the clinical trials didn't show a beneficial effect of immunosuppressive drugs. For example, prednisone and either azathioprine or cyclosporine were prescribed to 111 USA patients with histologically confirmed myocarditis but there was no improvement in transplant free survival or in left ventricular ejection fraction (LVEF) [35].

The treatment of chronic phase of viral myocarditis should be in combination between angiotensin and adrenergic pathway inhibition, such as eplerenone or spironolactone and diuretics for optimization of intravascular volume. Anticoagulants are required in patients with atrial fibrillation or with venous thromboembolism. Also, parenteral inotropes, including milrinone as an example for phosphodiesterase inhibitors or dobutamine as adrenergic agonists should be given to patients with severe myocarditis and symptomatic hypotension. Patients with ongoing systolic dysfunction are required in addition to consumption of therapy changing of their lifestyle such as, consumption of low-sodium diet, fluid restriction, and avoiding of non-steroidal anti-inflammatory drugs [31].

In addition to that arrhythmia suppression and hemodynamic support with vasopressors and positive inotropic agents may be necessary for patients with severe myocarditis. Persons with severe fulminant myocarditis may require aggressive short-term support with an intra-aortic balloon pump or left ventricular assist devices [4-6].

From previous studies we found an example of treatment of viral myocarditis that caused by coxsackievirus B3 (CVB3),

a member of enteroviruses, a positive single stranded non-enveloped RNA virus. About 30% of viral myocarditis cases are caused mainly by CV3 that enters cardiomyocytes through endocytosis via coxsackie and adenovirus receptor (CAR), the receptor which located in the same site with junction protein such as occludin [36,37].

When CVB3 particles attach to CAR, the conformation of the receptor will change forming a viral particle called A-particle, a product of the reaction occurs between the virus and CAR, this interaction permits the process of releasing of viral RNA inside the host cells and initiates viral transcription then translation. The observation of viral trapping by soluble CAR protein causing inactive A-particles proposed a strategy or a plane in CVB3 treatment. The observation that soluble CAR protein can function as a virus trap leading to inactive A-particles has suggested a strategy for CVB3 therapy [38-40].

However, the studies on viral myocarditis are continuous, no clinical trial is available until now, and managements with supportive treatment, such as improvement of hemodynamics with drugs and reducing of viral load by consumption of antiviral therapies still highly recommended [41-43].

Treatment trends (strategies) of myocarditis with CVB3 infection

- CVB3 infection treatment by nucleic acid-based antivirals.

The first strategy was based on antisense oligonucleotides (ASONs) against CVB3 which were designed to have the ability to interact with the complementary genetic segment in the target mRNA forming RNA-DNA heteroduplexes. The new sequences will be recognized by RNase H, which begin to digest only RNA strand in the hybrid RNA-DNA duplex. Because the instability, non-specificity, and undesired side effects of this molecule, ASONs, the structure has been changed in different structural parts such as nitrogen bases, ribose sugar, or phosphate group, then now it has been entered the new generation (the third generation) [44].

Induction of CpG containing oligodeoxynucleotide, was also used to activate antiviral immunity. The activation is due to the C type of CpG oligomer will activate response against the infection with CVB3 in human peripheral mononuclear cells by stimulation of releasing of interferons [45].

Regarding the treatment of CVB3, the second strategy has been proposed that the using of ribozyme as an antiviral (a small active RNA molecule that acts as enzyme and cleaves the single strand of RNA specifically with no need to proteins) has good therapeutic advantages. Ribozymes have the ability to transact and to confer specificity and virtually cleave any target sequence through fusion of the ribozyme core sequence at the 5’ and 3’ ends with the complementary sequences to the target gene [46]. This strategy has been proposed but not documented against CVB3 viral infection while many studies were reported against hepatitis C viral infection [47,48].

Using of RNA interference (RNAi) of CVB3 as a third strategy contributes successfully in the defense mechanism against viral infection in human cells [49].

The specific silencing of RNA is mediated by small types of RNAs the first termed short interfering RNAs (siRNA) and the second called microRNA (miRNA). The siRNA generated during the cleavage of double stranded non-coding RNA by one of III endoribonucleases class into siRNA (fragments with a 21-25 nts in length), these fragments are gathered into a complex, called the RNA-induced silencing complex (RISC), this complex will cause an insertion of a single strand of the siRNA as an antisense sequence to silence the target gene [50,51]. While miRNA is formed from the primitive sequences of non-coding regions (introns) of protein-coding polymerase II transcripts which called primary miRNA which

produced from a process of RNase III Drosha to produce about 70-nt long pre-miRNAs, which are transferred to the cytoplasm via exportin-5 and then cleaved by Dicer to be the activated miRNA. The role of these two types of RNA is based on the nature of interaction between the siRNA or miRNA and their interaction with the target sequences and this binding also depends on the complementarities between the miRNA and mRNA even it was complete or partial, this will lead to cleavage of the target sequence or inhibit the translation [52,53].

The strategy of Anti-CVB3 siRNAs is more efficient than Anti-CVB3 antisense oligonucleotides (ASONs) strategy because the inhibition of any genes of viral in addition of cells due to the effect of small double-stranded RNAs, as a silencer of target gene expression if the sequence of target gene is unique. From this reason the target search for anti-CVB3 siRNAs is concentrate on both CVB3 genome and the host cellular genes which are contribute and play a role in viral replication or viral infection. Viral genome carries multi cis-acting sequences required for viral transcription and translation processes, such as the 5' and 3' UTRs, IRES, and other sequences in the attachment sites of the transcription and translation initiation factors. Many genetic codes of the virus are encoding several necessary enzymes required for replication of the virus, such as proteases 2A and 3C in addition to polymerase 3D. All these structures are considered as suitable targets in designing of anti-CVB3 siRNAs. The earlier selection was concentrated on CVB3 protease 2A. Also, two independent groups approved that inhibition of 2A protease by a specific siRNAs will interrupt viral replicative cycle. In a previous study, five siRNAs revealed very strong activity against CVB3 in HeLa cells, by reduction about 92% of viral multiplication when targeting process of the 5' UTR, AUG start codon, VP1, 2A and 3D, respectively and found that the siRNA targeting 2A (nts 3543-3561) while siRNAs targeting VP1, 3D and the 5'UTR showed low antiviral effects, respectively. Furthermore VP1, a viral structural protein was chosen as a good target to evaluate anti-CVB3 siRNAs; however, informative data from previous studies didn't show active role of the siRNA targeting this gene in compare to other genes [54].

Regarding host cellular genes, CAR receptor was chosen to be a suitable target to inhibit both CVB3 and adenovirus because this receptor sharing the attractive site for these two viruses to cause myocarditis. A study was designed to silence the expression of CAR receptor with specific siRNAs through the transfection of siRNAs, siCAR2 or siCAR9 in HeLa cells, the complete silencing of the expression of CAR and also using of viral plaque assay showed approximately 60% reduction in the formation of CVB3 particle [55].

-Immunosuppression and immunomodulation therapies:

Immunosuppressive therapy continues to be investigated as a treatment for myocarditis. Although treatment of myocarditis is successful in many persons, some cases progress to congestive heart failure and may lead to a limited life span. For those persons, heart transplantation becomes an alternative therapy [8,9].

Immunosuppression therapies, such as cyclosporine and prednisone are suitable choice especially for giant cells myocarditis [56]. And muromonab 3-C also used as immunosuppressive drug and successfully treats the patients with giant cell myocarditis [57].

Mesenchymal stromal cells (MSCs) have cardioprotective and immunomodulatory properties [58,59]. *In-vitro*, coculture with CVB3-infected HL-1 cells, CVB3-induced apoptosis and consequent viral progeny release were reduced by MSC. *In-vivo*, intravenous injection of MSC improved the contraction and relaxation in myocarditis with CVB3 and caused a

reduction in cardiac apoptosis, cardiomyocyte damage, cardiac mononuclear cell activation, and cardiac fibrosis, with low reduction in CVB3 load [60,61].

Regulatory T cells (Treg) constitutes 5–10% of the peripheral T cells, and stated as regulator of the immune reaction [62], Treg is play an essential role in the induction and maintenance of immune homeostasis in viral myocarditis [63]. Studies in myocarditis declared that direct Tregs application might be a promised strategy in the treatment of myocarditis, this hypothesis is based on prophylactic [64,65] and therapeutic [66] adoptive transfer of Tregs which may improve CVB3 myocarditis [67].

Experimental studies on viral myocarditis showed a good result after the treatment by immunoglobulins (IgGs) as immune-omodulator therapy via the prevention of myocardial injury. IgGs also will reduce the scar formation and decrease the level of pro-inflammatory TNF-α and increase the anti-inflammatory interleukins IL-1 and IL-10 [68,69].

Antiviral chemotherapy:

Many antiviral agents were produced to inhibit one of the steps in viral replication, such as WIN 54954 which inhibits the interaction between the virus and host receptors [70].

Pleconaril is another example of antiviral therapy inhibits viral capsid integration. This drug integrates in viral capsid VP1 within a hydrophobic pocket leading to prevent either viral capsid attachment to host cells or inhibits viral uncoating [71].

In summary we conclude that viral infection that causing myocarditis may lead to subsequent autoimmune disease. Viral infections are one of the causes of the myocarditis that lead to different cardiomyopathy. It is necessary for all patients to receive standard care and treatment for heart failure as outlined in the ACC/AHA/ESC, and Heart Failure Society of America guidelines.

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