

New Strategies for the Treatment of Sick Sinus Syndrome

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ABSTRACT

Sick sinus syndrome (SSS) is an organic disorder of the sinoatrial node and its adjacent tissues, which causes problems in sinus node pacing function and sinus node conduction, thus leading to arrhythmias and multiple symptoms. The aetiology of the syndrome is complex, and the pathogenesis is unknown. Traditional therapies for SSS include drug therapy and pacemaker implantation, which have only partially succeeded clinically and cannot cure SSS. Hence, there is an urgent need to develop targeted therapies in order to treat SSS. This article aims to summarize the aetiology and pathogenesis

of SSS, discuss the advantages and disadvantages of traditional treatment methods, and focus on the biological pacing strategies based on gene and cell therapy.

Key words: Sick sinus syndrome; Pathogenesis; Traditional therapy; Gene therapy

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INTRODUCTION

Sick sinus syndrome (SSS), also known as sinus node dysfunction (SND), includes sinus arrest, sinoatrial block, bradycardia and tachycardia and other arrhythmia manifestations. The syndrome occurs at all ages, including newborns, with the highest incidence among people aged over 65 years. Previous population studies have shown that older adults are at increased risk of SSS^[1-3]. At present, SSS remains an incurable and progressive disease. Traditional treatment methods include drug therapy and nondrug therapy. Drug therapy includes the use of western medicine and traditional Chinese medicine to stabilize the heart rate. Nondrug therapy mainly involves the use of electronic pacemaker implantation. This article aims to summarize the aetiology and pathogenesis of SSS, discuss the advantages and disadvantages of traditional treatment methods, and focus on the biological pacing strategies based on gene and cell therapy.

CLINICAL MANIFESTATIONS AND AETIOLOGY

1. Clinical manifestations

The onset of SSS is insidious, and the course of development is mostly slow. It can take 5-10 years or even longer from the onset of symptoms to severe symptoms. The main clinical manifestation of SSS is bradycardia; as the disease progresses, cardiogenic cerebral ischaemic syndromes can appear, such as dizziness, darkening or syncope, and even SSS or sudden death. Cardiac abnormalities are characterized by palpitations, congestive heart failure and angina pectoris, and these three major cardiac symptoms are interrelated.

Oliguria, loss of appetite and muscle soreness also occur^[2].

1.2. Aetiology

SSS is not caused only by a single factor. Different pathophysiological mechanisms can cause similar disease phenotypes. Table 1 lists the causes of SSS. The causes of SSS are both endogenous and exogenous. Endogenous causes include sinoatrial node (SAN) degenerative fibrosis, coronary heart disease, cardiomyopathy, myocarditis, pericarditis, hypertension, invasive disease and congenital heart disease. Exogenous causes include drug effects (β -receptor blockers, calcium antagonists, digoxin and antiarrhythmic drugs), vagus nerve tension, electrolyte disturbance, surgical injury, hypothyroidism and increased intracranial pressure^[4-9]. The endogenous aetiology is usually irreversible; once it occurs, an electronic pacemaker is usually implanted. However, some exogenous causes of SSS are reversible. For example, the symptoms can be improved by discontinuing the patients' medication. SSS can also be familial, and mutations in genes such as *HCN4*, *SCN5A*, *MYH6* and *CACNA1C* can cause SSS^[10-14].

TRADITIONAL TREATMENT

SSS has no standard treatment. The clinical treatment principle is to accurately determine the cause of this condition and treat it symptomatically. For patients with SSS, the cause should be identified and corrected, the normal function of the sinus node should be restored, and further development of the disease should be prevented. There are two main types of clinical treatment for SSS: drug therapy and pacemaker implantation.

1. Drug treatment

At present, there is no clinical consensus on the optimal medication regimen for SSS. Therefore, drug treatment is often difficult, it lacks long-term therapeutic effects, and various antiarrhythmic drugs often have significant and intolerable side effects. It is necessary to determine the timing of treatment, control the dose of the medication, and avoid using medications that can slow down the heart rate (such as β -blockers, verapamil, digitalis and other drugs) during drug treatment. For patients with mild SSS, no special treatment is needed; only regular follow-up observations are required^[15]. When the patient develops bradycardia and obvious symptoms, conventional drugs (such as atropine, aminophylline, isoprenaline and nicotiny) can be used to increase the heart rate. Intravenous atropine and β -stimulants, although effective in the short term, are harmful when used long term. Their side effects include cardiotoxicity and ventricular arrhythmias^[16]. For patients with slow-fast syndrome, it is difficult to manage the bradycardia and tachycardia. Digitalis can be used in small doses when necessary to prevent or reduce the occurrence of tachyarrhythmia. Small doses of digitalis do not affect the sinus node and the atrioventricular conduction system. For neonatal patients in whom immediate implantation of a pacemaker is difficult, the results of a recent study^[17] suggest that procaterol is an effective treatment for infants and young children with SSS. After the diagnosis of SSS, medications were administered to the baby boy in a forementioned study through the gastrointestinal enteral route. The patient's condition was well controlled, and pacemaker implantation was no longer required. Cilostazol is effective for the treatment of symptomatic SSS and can stabilize the condition by increasing the heart rate, thereby preventing the need for permanent pacemaker implantation^[18]. In Japan, cilostazol has been widely accepted for the clinical treatment of bradycardia^[19]. In

Table 1 Causes of Sick Sinus Syndrome.

| Degenerative fibrosis | Pharmacologic agents | Familial SSS |
|------------------------------------|---------------------------------------|----------------------------|
| Aging | Digitalis | SCN5A, HCN4, MYH6, CACNA1C |
| Atrial tachyarrhythmias | Digoxin | |
| Associated with atrial myopathy | β -Blockers | |
| Amyloidosis | Calcium channel blockers | |
| Connective tissue diseases | Antiarrhythmics | |
| Hemochromatosis | Sympatholytics | |
| Sarcoidosis | Cimetidine | |
| Hereditary muscle dystrophies | Clonidine, methyldopa, reserpine | |
| Myocarditis | Lithium, phenothiazine, amitriptyline | |
| Valvular heart disease | Metabolic disorders | |
| Heart failure | Hyperkalemia | |
| Hypertension | Hypocalcemia | |
| Diabetes | Hypothermia | |
| Obesity | Hypoxia | |
| Obstructive sleep apnea | Acute ischemia | |
| Pediatric causes | Surgical injury | |
| Congenital abnormalities | | |
| Sinoatrial nodal artery deficiency | | |
| Infective | | |
| Rheumatic fever | | |
| Chagas disease | | |
| Diphtheria | | |

addition, traditional Chinese medicine and decoction treatment, and acupuncture and other methods have increasingly been recognized in clinical practice because of their significant effects and few adverse effects^[20]. Shenxian-shengmai oral liquid is a kind of Chinese patent medicine that is used in the treatment of patients with mild SSS^[21-24]. Traditional Chinese medicine research studies have shown that ginsenoside and calcium dibutyryl adenosine can effectively treat SSS^[25]. Although the efficacy of traditional Chinese medicine, acupuncture and other treatment methods in improving sinus node function was only reported in clinical studies, these methods were still applied in the clinical setting and was proven to be effective. However, pharmacological research on the drug and its mechanism of action are still in a fuzzy state.

2. Pacemaker implantation

Since the use of electronic pacemakers in the clinical setting^[26-27], several patients' lives have been saved. Permanent pacemaker implantation is currently the only effective treatment for SSS and the most common cause of pacemaker implantation^[28-29]. The pacemaker's battery is usually placed in the subcutaneous tissue of the chest or abdomen, and works through pulse generators and electrode wires^[20]. Despite the success in the use of electronic pacemakers, there are still disadvantages that cannot be ignored, including inability to adjust autonomously, limited battery life, lead displacement, possibility of infection and bleeding, and vulnerability to electromagnetic interference. Moreover, the use of pacemakers in paediatric patients remains a challenge^[26,30-32].

There has been controversy regarding the choice of pacemaker mode (single-chamber atrial pacing (AAIR) or dual-chamber pacing (DDDR)) in patients with SSS. Owing to the development of pathological SSS, atrioventricular block and atrial fibrillation

are common. Although a large number of studies have reported the advantages of single-chamber atrial pacing in patients with SSS, this method is rarely used. In daily clinical practice, because of the fear of high atrioventricular block after pacemaker implantation, in order to improve the prognosis, most patients with SSS choose a conventional dual-chamber pacemaker. Does every SSS patient need a dual-chamber pacemaker? Is it more reasonable to choose a dual-chamber pacemaker (DDDR) or a single-chamber pacemaker (AAI)? The DANPACE study is a multicentre randomized study conducted in Denmark. A total of 1,415 patients with SSS were randomly divided into an AAIR group and a DDDR group. The average follow-up was 5.4 ± 2.6 years. The results suggest that for SSS patients, when there is no significant difference in all-cause mortality when receiving AAIR and DDDR treatment, DDDR treatment should be preferred to reduce the risk of atrial fibrillation and re-pacing^[33]. However, recent studies^[34] have shown that AAIR pacing should be considered in patients with SSS without atrioventricular block. Therefore, for patients with SSS who require a permanent pacemaker implantation, an appropriate pacing method should be selected after the intracardiac electrophysiological examination. Patients who receive a permanent pacemaker are at a higher risk for atrial fibrillation; hence, frequent pacemaker examinations should be performed. Patients with a history of atrial fibrillation should also be assessed for the risk of stroke and bleeding, and make informed decisions about the use of anticoagulants.

NEW TREATMENT STRATEGIES

Drugs and electronic pacemakers have only achieved partial success in treating sick sinus syndrome. At present, there is an urgent need to develop targeted therapies based on pathogenesis to treat sick sinus syndrome. Recent studies have explored the feasibility and applicability of biological pacing, demonstrating the potential utility of gene- and cell-based therapy for sick sinus syndrome^[35-40]. SAN is the main pacemaker of the heart, and SSS is associated with structural and functional abnormalities of SAN. A better understanding of the genes that regulate the development and function of SAN is essential for the development of biological pacemakers. Therefore, before introducing biological pacing strategies, we first introduce tissue and organ lesions and the relationship between gene regulation and disease occurrence.

1. Tissue and organ diseases

SAN is the main pacemaker of the heart. It is located at the junction of the superior vena cava and the right atrium. It is centred on the SAN artery and can regulate the heart's beating frequency^[41]. SAN includes the head, centre, tail, peripheral areas and several sinus node conduction pathways (SACPs). The 'guide pacemaker' site, the first activated site of SAN, is usually located in the central part of SAN, and SACPs are considered to be the preferential conduction pathway of electrical impulses from SAN to the atrial myocardium^[42-45] (Figure 1). Sinus pacing and conduction abnormalities can cause arrhythmias, and sick sinus syndrome is associated with structural and functional abnormalities of SAN.

A mature SAN is composed of different types of cells, including cardiac pacing cells and a large number of nonpacing cells. Pacing cells are usually assigned into clusters surrounded by fibroblasts and a variable extracellular matrix (fibrous tissue). When the SAN pacing cells are gradually replaced by fibrous tissue, the number of pacing cells will decrease, and their normal pacing function will gradually decrease^[55,46-47]. With age, the increase in pathological fibrosis in SAN and SACPs interrupts the tight coupling between SAN myocardial



Figure 1 SAN is a multicompartment structure, composed of a head / centre and tail / periphery region, and several specialized conduction pathways. The schematic above represents 3D reconstruction of SAN (blue) with five SACPs (yellow). Modified from^[44].

cells and affects the interconnections between cells, which is essential for the stable pacing of SAN. This will usually result in bradycardia and conduction block. Sinus node fibrosis is involved in the pathogenesis of SSS^[3]; SAN tissue fibrosis affects the generation and conduction of SAN action potentials, leading to SSS^[5,48]. The transient receptor potential cation channel M7 (TRPM7) is associated with cardiac fibrosis. Zhong *et al.*^[49] found that SAN fibrosis is regulated by the Ang II/TRPM7/Smad pathway in SSS. *TRPM7* is a potential target for sinus node fibrosis treatment in SSS.

Changes in connexin expression in the SAN region accompanied by ageing are also associated with SND^[5]. Connexins form gap junctions between adjacent cells and play an important role in pulse propagation between cells and even throughout the heart^[50]. Compared with atrial tissues, several key ion channel protein subtypes were expressed in human SAN with a higher expression of connexin *Cx43*. This uneven expression promotes its autonomy and protects it from arrhythmias^[7,44].

In the course of cardiac surgery, when SAN or surrounding tissue is directly damaged, an abnormal sinus node function can occur after surgery and cause arrhythmia. Heart disease or immune disease may cause the SAN cells to gradually die due to ischaemia and hypoxia, eventually leading to the decline in SAN function and thus SSS^[51].

2. Gene regulation and disease occurrence

2.1. Transcription factors and SSS

The abnormal development of SAN will lead to the occurrence of SSS. Transcription factors related to the development of SAN include *Tbx18*, *Tbx3*, *Shox2*, *Isl1*, *Nkx2-5*, and *Pitx2*^[45,52-58]. *Tbx18* is an important transcription factor during embryonic development and plays an important role in the formation and development of SAN. *Tbx18* controls SAN head formation, but *Tbx18* deletion does not affect tail development. *Tbx3* is also expressed in SAN. During heart development, *Tbx3* is essential for pacemaker and conduction system development^[59]. During *Tbx18* controlling head formation, *Tbx3* further regulates the pacing gene program. *Shox2* is involved in the differentiation of pacing cells. During cardiac development, *Shox2* loss can cause sinus node developmental disorders, which is manifested by bradycardia. *Shox2* works by inhibiting *Nkx2-5* and simultaneously activating *Isl1* expression. The transcription factor *Isl1* plays a role downstream of *Shox2*, is necessary for SAN premitotic cell proliferation, and is expressed in both embryonic and adult SAN^[60-61]. Under normal circumstances, SAN is developed unilaterally, and *Pitx2* directly inhibits the expression of *Shox2* and upregulates two microRNAs that can inhibit the expression of the SAN gene^[58]. In *Pitx2*-deficient embryos, SAN develops bilaterally^[24]. The formation of SAN is strictly regulated by a network of transcription factors. These transcription factors present a dynamic and unique expression pattern in the working myocardium of SAN and its surrounding atria, in the differentiation and specialization of pacemaker cells, and in maintaining pacemaker characteristics and functions. However, under physiological and pathological conditions,

the role of these pacemaker-specific transcription factors in the structural and functional homeostasis of adult SAN still needs to be explored in more detail^[45].

2.2. Ion channel-related genes and SSS

The hyperpolarization-activated cyclic nucleotide-gated cation channel (HCN) is a kind of cyclic superfamily cation channel. This channel plays an important role in regulation of the special current existing in the SAN pacing— I_f (funny current)^[44,62]. When the SAN tissue, or other heart tissue lesions containing HCN channel subtypes, is damaged, the HCN channel subtypes are abnormally expressed, failing to form I_f currents and eventually leading to arrhythmias^[63]. There are four subtypes of HCN channels (*HCN1–4*). *HCN1*, *HCN2* and *HCN4* are expressed in mammalian hearts, of which *HCN4* is the most important and most widely expressed, accounting for about 75% of I_f in SAN^[11,64–65]. *HCN4* is the main channel subtype of I_f that maintains the normal pacing current of the heart, and its deletion (heterozygous or homozygous) can cause embryonic pacing cells to fail or mature^[66]. Induction of *HCN4* knockout later in life in mice can lead to severe bradycardia and conduction system disease, suggesting that the gradual loss of *HCN4* channel function over time may contribute to the development of SSS^[67]. Previous studies have reported several mutations in the *HCN4* gene, and these mutations affect the normal expression of HCN4 protein, leading to familial SSS^[63,68–73].

The voltage-gated sodium channel (Nav) and the I_{Na} current generated play an important role in the promotion of membrane potential depolarization. Among them, the *SCN5A* gene encoding the α -subunit of the Nav1.5 ion channel has been extensively studied, because it plays a key role in cardiac excitability. This gene mutation is related to a variety of human diseases and often causes severe arrhythmias, including atrial fibrillation, premature beats and SND^[74]. So far, a large number of studies have found that the *SCN5A* gene mutations can trigger human SSS^[75–78].

In addition, the L-type calcium channel current ($I_{Ca,L}$), which relies on the formation of L-type calcium ion channels, can be transmitted to all regions of the heart and is an extremely important inward current during the diastole of SAN cells. In SAN, Cav1.2 and Cav1.3-type calcium channels play an important role^[11,79]. The gene mutations in the calcium channel subunits *CACNAID* and *CACNAIC* can cause SSS^[12,80].

2.3. MicroRNA and SSS

MicroRNA (miRNA) is an endogenous, short, noncoding RNA that mediates gene silencing at the post-transcriptional level^[81]. miRNAs can cause a variety of diseases, including heart failure, myocardial hypertrophy and myocardial infarction^[82–83], all of which may increase the susceptibility to SSS. For example, *miR-17-92* and *miR-106b-25*, which are positively regulated by the transcription factor *Pitx2*, can directly target the inhibition of *Shox2* and *Tbx3* genes, which can promote SAN cell differentiation. In animal experiments, SND was found in both heart-specific knockout *miR-17-92* mice and *miR-106b-25* heterozygous mice^[58]. miRNAs can regulate cardiac ion channel gene expression at the post-transcriptional level^[84–85]. Recent research^[79] has found that *miRNA-1976* can play an important role in SAN ageing by targeting *Cav1.2*- and *Cav1.3*-type calcium channels. Therefore, *miRNA-1976* is a potential noninvasive diagnostic tool and therapeutic target for SSS.

3. Biological pacing therapy

3.1. Biopacing based on gene therapy

Gene biopacing refers to the use of genetic engineering technology to introduce the target gene into the damaged autonomic rhythm point or the tissue of the conduction system. By introducing the gene to express the corresponding protein or inhibit the expression of certain genes in the body, the normal cardiac pacing or conduction function will be restored.

Pacing-like cells can be induced by transcription factor recoding^[86–88]. For example, the *TBX18* gene, which is essential for the development of the SAN head, is an important gene that can induce the production of pacing-like cells. Kapoor *et al.* [87] demonstrated the transformation of rodent cardiomyocytes into SAN cells in vitro and in vivo through the expression of *TBX18*. *TBX18* was overexpressed in adult guinea pig myocardium, and transfected cardiomyocytes showed morphological and electrophysiological characteristics of SAN cells. Hu *et al.*^[89] used a minimally invasive method to inject a viral vector carrying *TBX18* into a pig heart to develop a ‘biological pacemaker’ that can treat heart rhythm abnormalities, and they proved that the pacemaker is sufficient to support daily life needs. Overexpression of the *TBX18* gene enhances the function of the atrial pacemaker in SSS rats, suggesting that the gene therapy targeting *TBX18* may be integrated in human sick sinus nodes^[90,91]. The pacemaker function was restored in the affected patient, thereby avoiding the need for an electronic pacemaker. Bakker *et al.*^[86] constructed *Tbx3*-overexpressing mice and isolated adult mouse cardiomyocytes for analysis. They found that the working myocardium had effectively transformed into pacemaker cells, and the expression of related genes encoding connexin and cardiac sodium channels was all suppressed. Although the results of reprogramming based on transcription factors are encouraging, two points are worth noting: one is the heterogeneity and the other is the limited duration of the phenotype after successful reprogramming. However, these problems may be solved by the long-term overexpression of nonimmunogenic vectors such as the adeno-associated virus AAV.

Biological pacing can be achieved by overexpressing ion channel-related genes in vivo^[92,93]. For example, the role of HCN channel overexpression in the myocardium has been widely studied as a biological pacemaker strategy with some success^[35,94,95]. Morris^[96] and others found that the use of adenovirus-mediated overexpression of HCN2 and HCN212 genes can accelerate the pace of atrial-assisted pacemakers. This study provides theoretical proof for a new biological pacing strategy. The approach will help treat bradycardia in patients with SSS.

3.2. Cell-based biopacing

Cell-based artificial biological pacemakers can use stem cells to induce pacing-like SAN cells. They can be divided into embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) and mesenchymal stem cells (MSCs) according to their source. In addition, cell therapy methods include the use of cells as a delivery tool for gene construction^[97,98]. At present, gene transfection vectors mainly include adenovirus, adeno-associated virus, lentivirus, liposome and electroporation. Among them, the most commonly used is adenovirus.

ESCs and iPSCs can differentiate into cardiomyocytes, can show intrinsic pacemaker activity, and can pace ventricular muscle in vivo^[99–102]. Mummery *et al.*^[103] used human ESCs to induce differentiation in the cardiac muscle tissue with pacing function. Zhu *et al.*^[104] found that the intervention of the NRG-1 β /ErbB signalling pathway in ESCs can increase the expression of *Tbx3*, thereby increasing the expression of sinus node-related genes, promoting the

differentiation of stem cells into sinus node-like cells, and detecting sinus node-like action potential. However, the application of ESCs in clinical practice is facing ethical issues; hence, their clinical application is limited. iPSCs have pluripotency similar to ESCs, and their differentiation potential is similar to ESCs. The method used to obtain them is relatively simple and stable. They can be obtained from autologous cells without the need for egg cells or embryos. This prevents immune rejection and ethical controversies in cell transplantation. Spontaneous beating cells are present in iPSC-derived cardiomyocytes (iPSC-CMs), and diastolic depolarization potentials and genes associated with sinus node characteristics can be detected^[105,106]. Recent studies^[107] have found that TBX3 can induce pacing function in human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). However, TBX3 overexpression alone is not enough to cause the diastolic depolarization of hiPSC-CMs, whereas the simultaneous overexpression of TBX3 and HCN2 can successfully re-encode hiPSC-CMs into pace-like cells and cause diastolic depolarization. This provides a new strategy for building biological pacemakers.

MSCs are pluripotent stem cells with promising prospects in the field of regenerative medicine. This and other studies have shown that overexpressing human genes such as *pygo2* with *Nkx2.5* to promote the differentiation of human umbilical cord MSCs into cardiomyocytes is a better method to obtain cardiomyocytes^[108-109]. Other studies have shown^[110] that 5-azacytidine-induced bone marrow MSCs can differentiate into pacemaker-like cells that express HCN2 channel proteins and *I_f* currents. In addition to chemical induction, microenvironment induction can be adopted. The microenvironment induction method is safer and prevents the risk of cytotoxicity and mutagenicity. Wang *et al*^[111] found that bone marrow MSCs can interact with cardiomyocytes and express connexins Cx40 and Cx43, forming gap junctions and open HCN channels, generating pacing function, and maintaining stability for a long time. MSCs can be obtained *in vivo*; are easy to isolate, purify, expand and culture, without the risk for immune rejection and ethical issues; and easy to integrate foreign genes. At present, most studies have used MSCs as a carrier, and transfection pacemaker genes or transcription factors that regulate the differentiation of SAN obtain sinus node-like cells that can express the pacing gene to produce current characteristics. MSCs have become the ideal carrier cells for the biological pacing treatment of SSS. Yang *et al*^[112] used the HCN4 lentiviral vector to transfect MSCs, the pacing-like cells expressed the typical pacing protein HCN4, and the whole patch clamp detected a typical pacing current *I_f*.

CONCLUSION

At present, neither the medical treatment nor the implantation of artificial pacemakers can cause physiological or pathological changes to the heart. The development of a biological pacing treatment strategy brings new hope for the cure of SSS patients; however, there are still other techniques of using biological pacing in clinical practice. Several issues such as the selection of biological pacing target genes, a safe, effective and controllable *in vivo* gene delivery system, and the safety of stem cells have not been completely resolved. The replacement of electronic pacemakers by biological pacemakers is still the direction of future development. Hence, researchers should continue to use various animal models for experimental research in the molecular field, explore the pathogenesis of new SSS, and improve their understanding of the pathogenesis of SSS in order to discover new therapeutic targets. In the near future, through continuous research, biological pacemakers can be safely

and effectively used in the treatment of patients with SSS. To date, electronic pacemakers remain the only effective treatment for SSS. Before biological pacemaker therapy is successfully applied in the clinical setting, older adults should take precautions according to their cause of illness. Moreover, prognosis management should be implemented in sick people.

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