THERAPEUTIC EFFECTS OF HUMAN PERICARDIAL FLUID CELLS IN DOXORUBICIN-INDUCED HEART FAILURE RAT MODEL VIA INTRAPERICARDIAL INJECTION

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LIST OF ABBREVIATIONS

ACEI	Angiotensin-converting enzyme inhibitors
Ang II	Angiotensin II
APC	Allophycocyanin
ARBs	Angiotensin receptor blockers
ADSCs	Adipose-derived stem cells
Ca^{2+}	Calcium
cCFU-F	Colony-forming unit-fibroblast
CD90	Cluster differentiation 90
cGMP	Cyclic guanosine monophosphate
CO	Cardiac output
Col I	Collagen I
Col III	Collagen III
CRT	Cardiac resynchronization therapy
CSA	Cross-sectional area
CSCs	Cardiac stem cells
cTnT	Cardiac troponin T
CVD	Cardiovascular disease
CVF	Collagen volume fraction
Cx43	Connexin-43
DAB	3,3-Diaminobenzidine
DOX	Doxorubicin
EPCs	Endothelial progenitor cells
EF	Ejection fraction
ESCs	Embryonic stem cells
FITC	Fluorescein isothiocyanate
Fzd 1-10	Frizzled proteins
HF	Heart failure
HE	Haematoxylin-esolin
hPFCs	Human pericardial fluid-derived cells
HR	Heart rate
HSCs	Human hematopoietic stem cells

HSF-1	Heart shock factor-1
ICD	Implantable cardioverter-defibrillator
IGF-1	Insulin-like growth factor-1
IL-1	Interleukin-1
IL-6	Interleukin-6
IL-10	Interleukin-10
iPSCs	Induced pluripotent stem cells
Klf4	Kruppel-like factor 4
LV	Left ventricle
LVAD	Left ventricular assist device
LVEDV	Left ventricular end-diastolic volume
LVEF	Left ventricular ejection fraction
LVESV	Left ventricular end-systolic volume
LVFS	Left ventricular fractional shortening
MSCs	Mesenchymal stem cells
mtDNA	Mitochondrial DNA
NO	Nitric oxide
NF-κB	Nuclear factor kappa B
O.C.T. compound	Optimum cutting temperature compound
Oct3/4	Octamer-binding transcription factor 3/4
OM	Omecamtiv mecarbil
P (number)	Passage (number)
PBS	Phosphate-buffered saline
PCI	Percutaneous coronary intervention
PDGF	Platelet-derived growth factor
PE	Phycoerythrin
PEPCs	Pericardial effusion progenitor cells
QRS	Quality, rest, and strength
RAAS	Renin-angiotensin-aldosterone system
RFP	Red-fluorescence protein
ROS	Reactive oxygen species
SCA-1	Stem cell antigen 1
SD rats	Sprague Dawley rats
SEM	Standard error of mean

sGC	Soluble guanylate cyclase
SGLT2 inhibitors	Sodium-glucose cotransporter 2 inhibitors
S-ICD	Subcutaneous implantable defibrillators
SNS	Sympathetic nervous system
SOX-2	Sry-box transcription factor 2
SSEA	Stage-specific embryonic antigens
SV	Stroke volume
TDGF-1	Teratoma-derived growth factor 1
TGF-β	Transforming growth factor-β
TNF-α	Tumor necrosis factor-α
TV-ICD	Transvenous implantable cardiodefibrillators
VEGF-A	Vascular endothelial growth factor A
α-SA	α-skeletal actin
α-SMA	α-smooth muscle actin

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KESAN TERAPEUTIK SEL CECAIR PERIKARDIUM MANUSIA DALAM MODEL TIKUS KEGAGALAN JANTUNG YANG DIARUH OLEH DOXORUBICIN MELALUI PENYUNTIKAN INTRAPERIKARDIUM

ABSTRAK

Kegagalan jantung (HF) adalah punca utama kematian di seluruh dunia. Terapi sel telah muncul sebagai strategi alternatif untuk merawat HF. Kajian ini bertujuan untuk mengasingkan dan mencirikan sel daripada cecair perikardium manusia (hPFCs) serta menguji penempelan, kesan terapeutik, dan mekanisme tindakan sel tersebut selepas disuntik secara intraperikardial ke dalam model tikus HF aruhan doksorubisin (DOX). hPFCs telah diasingkan dan dikultur daripada cecair perikardium lima orang pesakit HF yang menjalani pemindahan jantung. hPFCs yang dikultur tersebut menunjukkan ekspresi penanda sel stem NANOG+/SCA-1+ (94.0% ± 1.8%), C-KIT$^+$/NANOG$^+$ (30% ± 4.0%), C-KIT$^+$/SCA-1$^+$ (16.6% ± 4.4%), dan CD90$^+$/CD105$^+$ (76.8% ± 15.5%) pada *passage* ke-3. Selain itu, ia juga menonjolkan penanda hematopoietik CD45$^+$CD90$^+$ (35.1% ± 19.7%) dan CD34$^+$CD73$^+$ (13.9% ± 6.9%). hPFC ini juga dapat diaruh secara *in vitro* untuk menjadi sel adiposit (70.6% ± 1.2%), sel osteoblas (29.3% ± 3.3%), dan sel kardiomiosit (81% ± 1.6%). DOX digunakan untuk mengaruhkan HF pada tikus selama 6 minggu (2.0 mg/mL). Ciri-ciri HF disahkan pada minggu ke-4 dengan pemerikasaan perubahan fungsi jantung, fibrosis, dan ekspresi sitokin inflamasi serta faktor angiogenik sebelum penyuntikan hPFCs (2.5×10^5 sel/jantung) *passage* ke-3 secara intraperikardial pada tikus tersebut. Parameter yang sama telah diuji sekali lagi selepas 1 dan 4 minggu suntikan sel tersebut. Suntikan intraperikardial hPFC telah menambahbaikkan kadar denyutan jantung, isipadu strok keluaran jantung, pecahan ejeksi ventrikel kiri, dan pemendekan

pecahan secara signifikan pada tikus HF aruhan DOX selepas 1 dan 4 minggu suntikan sel tersebut. Begitu juga dengan isipadu akhir diastolik dan isipadu akhir sistolik ventrikel kiri dalam kedua-dua kumpulan rawatan yang berkurangan dengan signifikan pada 1 minggu (219.8 ± 6.2 μl, P=0.0229; 100.5 ± 2.9 μl, P<0.0001) dan 4 minggu (171.4 ± 7.4 μl; 63.2 ± 13 μl, P<0.0001) berbanding dengan kumpulan kawalan tanpa rawatan yang menunjukan peningkatan isipadu-isipadu tersebut (257.6 ± 14.3 μl; 166.7 ± 10.5 μl). Sel hPFC yang berlabel red fluorescent protein (RFP) juga dijejak selepas 4 minggu suntikan dan didapati sel hPFCs berlabel RFP ini telah mengekspresikan troponin T jantung dan konnesin 43 dalam miokardium. Pemerhatian ini sejajar dengan pengurangan yang signifikan dalam fibrosis (P<0.0001) dan sitokin inflamasi IL-6, IL-10, dan TNF-α dalam jantung selepas rawatan hPFCs (P<0.001) berbanding dengan kumpulan tanpa rawatan. Kumpulan rawatan hPFCs juga telah menunjukkan peningkatan dalam faktor angiogenik VEGF-A selepas 4 minggu (P<0.05 berbanding dengan kumpulan tanpa rawatan). Oleh itu, suntikan hPFCs melalui perikardium dapat memperbaiki fungsi jantung tikus HF aruhan DOX secara signifikan. Mekanisme asas mungkin berkait rapat dengan pengurangan fibrosis jantung, penekanan tindak balas radang, dan peningkatan paras faktor angiogenik VEGF-A. Hasil ini mencadangkan bahawa suntikan intraperikardium hPFCs bukan sahaja berkesan memperbaiki manifestasi klinikal HF tetapi juga mungkin memberikan strategi terapeutik baharu untuk pembaikan jantung.

THERAPEUTIC EFFECTS OF HUMAN PERICARDIAL FLUID CELLS IN DOXORUBICIN-INDUCED HEART FAILURE RAT MODEL VIA INTRAPERICARDIAL INJECTION

ABSTRACT

Heart failure (HF) is the leading cause of mortality worldwide. Cell therapy has emerged as an alternative strategy for treating HF. This study aimed to isolate and characterize human pericardial fluid-derived cells (hPFCs) and investigate the cell engraftment, therapeutic effects, and mechanisms of action after intrapericardially administered into a doxorubicin-induced HF rat model. HPFCs were isolated from the pericardial fluid of five HF patients who underwent heart transplantation and cultured. The cultured hPFCs showed the expression of stem cell markers $NANOG^+/SCA\text{-}1^+$ (94.0% ± 1.8%), $C\text{-}KIT^+/NANOG^+$ (30% ± 4.0%), $C\text{-}KIT^+/SCA\text{-}1^+$ (16.6% ± 4.4%), and $CD90^+/CD105^+$ (76.8% ± 15.5%) at passage 3. Additionally, hemopoietic markers $CD45^+CD90^+$ (35.1% ± 19.7%) and $CD34^+CD73^+$ (13.9% ± 6.9%) were also expressed. These hPFCs were also able to differentiate into adipocytes (70.6% ± 1.2%), osteoblasts (29.3% ± 3.3%), and cardiomyocytes (81% ± 1.6%) *in vitro*. Doxorubicin (DOX) was used to induce HF in rats for 6 weeks (2.0 mg/mL). HF characteristics were confirmed at week 4 by examining the changes in cardiac function, fibrosis, and the expression of inflammatory cytokines and angiogenic factors prior to administering hPFCs (2.5×10^5 cells/heart) intrapericardially at passage 2. The same parameters were re-assessed for changes after 1 and 4 weeks of cell administration. Intrapericardial injection of hPFCs significantly improved heart rate, cardiac output stroke volume, left ventricular ejection fraction, and fractional shortening in doxorubicin-induced HF rats after 1 and 4 weeks. Similarly, the elevated left

ventricular end-diastolic (257.6 ± 14.3 μl) and end-systolic volume (166.7 ± 10.5 μl) in the untreated group were both significantly reduced with hPFCs treatment at 1 week (219.8 ± 6.2 μl, P=0.0229; 100.5 ± 2.9 μl, P<0.0001, respectively) and 4 weeks (171.4 ± 7.4 μl; 63.2 ± 13 μl, P<0.0001 versus untreated group). Red fluorescent protein (RFP)-labeled hPFCs were tracked after 4 weeks of injection, and these RFP cells coexpressed cardiac troponin T and connexin 43 in the host myocardium. This observation coincided with a significant reduction in cardiac fibrosis after hPFC treatment (P<0.0001 versus untreated group) and reduced the level of DOX-induced increased inflammatory cytokines IL-6, IL-10, and TNF-α in the heart following hPFCs treatment (P<0.001 versus untreated group). The hPFCs treatment group showed an increase in local angiogenic factor VEGF-A after 4 weeks (P<0.05 versus untreated group). Therefore, intrapericardial injection of hPFCs can significantly improve cardiac function in doxorubicin-induced HF rats. The underlying mechanisms may be closely related to the significant reduction of cardiac fibrosis, suppression of inflammatory responses, and increased levels of the angiogenic factor VEGF-A. These results suggest that intrapericardial injection of hPFCs not only effectively improves the clinical manifestations of HF but may also provide a novel therapeutic strategy for cardiac repair.

CHAPTER 1

INTRODUCTION

One of the most common chronic illnesses is heart failure (HF), which is an advanced stage or severe manifestation of a number of different cardiac problems. High rates of mortality and readmission are its defining characteristics (Dharmarajan et al., 2017). HF has become a major global health challenge, with approximately 64 million people affected worldwide. As the population ages, this number is expected to continue rising (Savarese et al., 2022). The prognosis for patients with HF is still dire despite tremendous breakthroughs in treatment. Existing standard treatments, including medication and lifestyle changes, can effectively alleviate symptoms, but in the long term, they are insufficient to fundamentally address the issue of cardiac function decline (Gorodeski et al., 2018). Heart transplantation and mechanical circulatory assistance are the main therapies for advanced HF. Nevertheless, patient eligibility, donor availability, and high prices limit these approaches (Sánchez-Enrique et al., 2017). As a result, investigating possible HF causes and creating new treatment targets is imperative (Metra and Teerlink, 2017).

Nowadays, cell therapy has become a highly promising strategy for treating advanced HF and has achieved many encouraging results in clinical research (Narita and Suzuki, 2015, Kobayashi and Suzuki, 2018). By injecting stem cells into the infarcted area, these cells can migrate to the damaged myocardium and accelerate the recovery of cardiac function through anti-apoptotic, immunomodulatory, and angiogenic effects (Roshanbinfar et al., 2021). Specifically, stem cells can inhibit cardiomyocyte apoptosis, reduce cell damage in the infarcted area, and thereby help maintain the structural integrity of heart tissue (Abdelwahid et al., 2016). Meanwhile, stem cells alleviate excessive inflammation by secreting anti-inflammatory cytokines,

preventing further fibrosis and cardiac injury (Vadivel et al., 2020). Additionally, stem cells promote the transformation of immune cells into reparative macrophages, enhancing the myocardial repair process (Xiong et al., 2021). By secreting angiogenic factors, stem cells also promote the formation of new blood vessels, improving blood supply to the infarcted area and ensuring effective delivery of oxygen and nutrients, thus facilitating the recovery of cardiac function (Jahani et al., 2020). The selected candidate cells should be able to provide cardiac-protective paracrine factors, induce local perfusion, and repopulate damaged myocardium with functional myocardial cells. Compared with traditional treatment methods, stem cell therapy has significant advantages in improving heart function and reducing inflammation (Wang et al., 2024). However, stem cell therapy also carries certain potential risks. First, stem cell transplantation may trigger immune rejection (Haworth and Sharpe, 2021). Second, due to the pluripotency of stem cells, tumorigenicity is an important risk, particularly in the interaction between the stem cells' differentiation potential and the transplantation environment (Deng et al., 2018). Additionally, cell survival and integration remain uncertain; transplanted cells may not survive long-term within the heart tissue or may fail to exert the expected reparative effects (Zhu and Cheng, 2021). Finally, stem cell transplantation may lead to arrhythmias, especially in the damaged areas of the heart, potentially causing electrophysiological disturbances (Chen et al., 2020).

Nevertheless, there are still many unsolved mysteries regarding the feasibility and effectiveness of cell therapy for patients with HF. One of these is the potential and effectiveness of using cells derived from the pericardial cavity to treat HF patients. Due to its proximity to cardiac tissue, the pericardial fluid inside the pericardium contained various cell types, including mesothelial cells, neutrophils, monocytes, lymphocytes, and other white blood cells, along with water and various soluble molecules (Gibson

and Segal, 1978, Benhaiem-Sigaux et al., 1985). While there are multiple sources of cells used for the treatment of cardiovascular diseases, cells derived directly from the heart have unparalleled advantages in treating myocardial injury due to their homology with the myocardium and close proximity to myocardial tissue during embryonic development. First, cells derived from the heart are highly similar to myocardial tissue at the genetic and molecular levels, making it easier for them to successfully integrate and function within the heart (Meilhac and Buckingham, 2018). Secondly, these cells are closely associated with heart tissue during embryonic development and inherently possess cardiac-specific functions, such as electrical conduction and muscle contraction (Moorman and Christoffels, 2003). More importantly, heart-derived cells are generally less likely to trigger immune rejection, making them safer for clinical applications (Menasché and Vanneaux, 2016).

Therefore, it is hypothesized that hPFCs offer great potential in treatment of HF. Thus, the project aimed to study the characteristics, cell retention, therapeutic effects, and mechanisms of hPFCs in a rat model of doxorubicin-induced HF. For that, human pericardial fluid was collected from HF patients. Subsequently, the hPFCs were isolated and cultured to evaluate their characteristics and cell differentiation potency. Finally, an HF rat model using doxorubicin (DOX) was established to explore the role of the isolated hPFCs in treating HF.

1.1 General Objective

The main objective of the study was to characterize and investigate cell retention, therapeutic effects, and mechanism of intrapericardially administrated hPFCs in doxorubicin-induced HF rat model.

1.2 Specific Objectives

1. To isolate and culture hPFCs harvested from HF patients and characterize their biochemical markers (such as C-KIT, SCA-1, and NANOG) and cell differentiation potential, including adipogenic, osteogenic, and cardiomyogenic differentiation abilities.
2. To establish a doxorubicin-induced HF rat model and assess cardiac function, histopathological changes, and molecular biomarkers associated with HF, such as Col I, Col III, IL-6, IL-10, and TNF-α.
3. To evaluate the cell retention and therapeutic effects of intrapericardially administrated hPFCs in a DOX-induced HF rat model, including tracking cell survival , as well as assessing improvements in heart function, myocardial injury, and cardiac fibrosis.
4. To investigate the underlying mechanisms of hPFCs in modulating inflammatory responses and regulating molecular markers associated with HF, including Col I, Col III, IL-6, IL-10, TNF-α, and VEGF-A.

1.3 Problem Statement

HF is a prevalent chronic illness characterized by elevated risks of death and readmission. Despite progress in treatment, the prognosis for patients remains poor. Mechanical circulatory support and heart transplantation are limited by patient qualifications, donor availability, and costs, making it necessary to explore new treatment methods. Nowadays, cell therapy, as a promising strategy for treating advanced HF, has shown encouraging results in clinical studies. The biological entities that have been explored the most for cell therapy are stem cells. However, their application faces several challenges, including determining the optimal cell delivery

pathway, ensuring cell survival post-administration, achieving adequate cell retention at the targeted site, enhancing therapeutic efficacy at the targeted site, and mitigating host rejection or immune response to the administered cells. In light of these limitations, we hypothesized that cells isolated directly from the pericardial cavity (referred to as hPFCs) may offer superior therapeutic potential compared to stem cells. hPFCs possess unique advantages in the treatment of myocardial injury, attributable to their homology with myocardial tissue and their anatomical proximity to the myocardium. This inherent compatibility may enhance their therapeutic efficacy and reduce the incidence of adverse immune responses. However, research on the potential and mechanisms of hPFCs in treating HF is still limited. Specifically, it is unclear how hPFCs interact with myocardial tissue in heart failure models and whether they can effectively promote myocardial repair or regeneration. Additionally, the long-term survival of hPFCs, their retention in the damaged myocardial area, and whether they can exert lasting effects in therapy remain unresolved questions. Thus, the study aimed to explore the characteristics, cell retention, therapeutic effect, and mechanism of hPFCs in a rat model of doxorubicin-induced HF.

CHAPTER 2 LITERATURE REVIEW

2.1 Heart Failure

HF is a complex clinical illness that typically manifests as pulmonary or systemic congestion and can result from a number of structural or functional heart disorders (Weintraub et al., 2010). It represents an advanced stage of various heart conditions, characterized by high mortality rates. In Malaysia, the epidemiological characteristics of HF patients indicate a relatively younger population with a predominance of males, and ischemic heart disease is the primary etiology. A 3-year prospective observational study revealed that among 2,717 patients hospitalized for acute HF, the average age was 60.2 ± 13.6 years, with 66.8% being male and 34.3% presenting with new-onset HF (Wan Ahmad et al., 2024). At admission, 55.7% of patients were classified as New York Heart Association (NYHA) Class III or IV, with ischemic heart disease being the most common cause, accounting for 63.2%. The hospitalization rate was 87.3%, and the in-hospital mortality rate was 2.9%. There are acute and chronic stages of HF, with the latter being a common cause of death from cardiovascular disorders (Tanai and Frantz, 2015). As the average human lifespan extends, the challenge of an aging population becomes more pronounced, contributing to an escalating incidence of HF. This issue has emerged as a significant global concern in the 21st century, demanding urgent attention.

Two common classification systems for HF: NYHA functional classification and the American College of Cardiology/American Heart Association (ACC/AHA) staging system (Bozkurt et al., 2021). The NYHA system is based on the subjective assessment of the patient's symptoms by healthcare professionals and classifies patients into four levels (Rohde et al., 2023):

Class I: No symptoms of HF.

Class II: Symptoms of HF with moderate exertion, such as walking two blocks or climbing two flights of stairs.

Class III: Symptoms of HF with minimal exertion such as walking one block or one flight of stairs, but no symptoms at rest.

Class IV: Symptoms of HF at rest.

The ACC/AHA staging system divides patients into four different stages based on their HF risk factors, cardiac structural abnormalities, and whether HF symptoms are present (Chairy, 2022).

Stage A: At high risk for HF but without symptoms, structural heart disease or cardiac biomarkers of stretch or injury.

Stage B: Pre-HF, defined as no signs or symptoms of HF but evidence of one of the following: structural heart disease, evidence of increased filling pressures or risk factors plus increased levels of BNPs or persistently elevated cardiac troponin.

Stage C: Structural heart disease with prior or current symptoms of HF.

Stage D: Marked HF symptoms that interfere with daily life and with recurrent hospitalizations despite attempts to optimize guideline-directed medical therapy (GDMT).

The NYHA functional classification focuses on assessing the severity of symptoms in patients, allowing for movement between different levels, while the ACC/AHA staging system emphasizes pathological progression, with a one-way progression where the staging can only move from Stage A to Stage D.

2.2 Pathophysiology of Heart Failure

The pathological development of HF typically involves multiple factors. The pathogenic mechanisms can be categorized into the following areas: nervous system

activation, inflammation and oxidative stress, mitochondrial damage, intracellular calcium ion (Ca^{2+}) overload, iron free radical production, and myocardial cell damage and remodeling (Tanai and Frantz, 2015). While there may be additional contributing factors, the primary pathophysiological mechanisms of HF are known to include nervous system activation, myocardial cell damage and remodeling, as well as inflammation and oxidative stress. These mechanisms interact synergistically, collectively advancing the progression of HF (Figure 2.1).

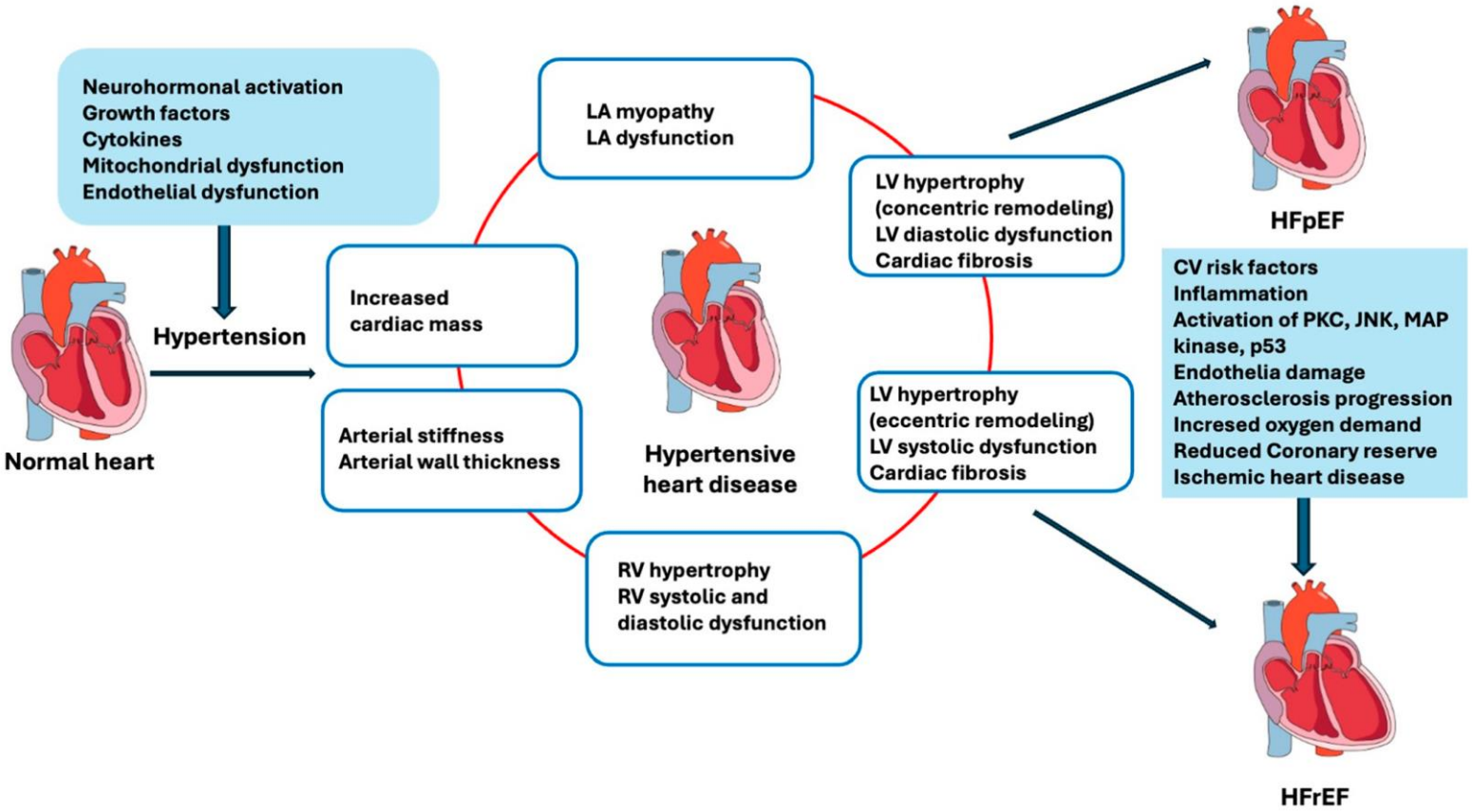

Figure 2.1 Progression of HF

The activation of neurohormones, growth factors, cytokines, mitochondrial dysfunction, and endothelial dysfunction interact with each other, collectively promoting cardiac remodeling and fibrosis, which in turn leads to a decline in cardiac function. These changes result in both left and right ventricular systolic and diastolic dysfunction, ultimately exacerbating the onset and progression of HF.

2.2.1 Neurohormonal Activation

Because of the decreased cardiac output during HF, the sympathetic nervous system (SNS) is instinctively engaged. The SNS exerts its effects primarily through β1, β2, and α1 receptors (Cheng et al., 2016). The binding of these receptors damages the myocardium, leading to compensatory blood flow to the failing heart. This results in an accelerated heart rate and increased myocardial contractility, ultimately causing an elevation in cardiac output. Over time, excessive SNS activation leads to myocardial compensatory hypertrophy, myocardial cell apoptosis, necrosis, and myocardial interstitial collagen deposition. Clinical manifestations of this process include decreased ejection fraction and arrhythmias (Hartupee and Mann, 2017). Moreover, renin-angiotensin-aldosterone stimulation (RAAS) can be triggered by activating β1 and α1 receptors, increasing the release of angiotensin II (Ang II). This exacerbates ventricular afterload, leading to ventricular remodeling. There is a close correlation between RAAS and SNS; the SNS stimulates the release of renin in RAAS, and Ang II also stimulates the SNS, fostering the release of aldosterone and vasopressin. Hyperaldosteronism resulting from this cascade can lead to autonomic dysfunction, reducing both sympathetic and parasympathetic activity (Davis et al., 1964). Ang II, in addition to being a vasoactive hormone, has a negative inotropic effect on heart (Dendorfer et al., 2002). It can directly induce myocardial cell hypertrophy and cause cell apoptosis (McEwan et al., 1998).

2.2.2 Myocardial Injury and Remodeling

When cardiac pressure or volume overload occurs, the initial response is myocardial cell hypertrophy, accelerating the cycle of myocardial cell apoptosis and regeneration, ultimately contributing to myocardial structural remodeling (Wu et al., 2017). In cases of myocardial injury, the activation of neurohormones triggers the

release of angiotensin II and endothelin-1, or vasopressin, which, in turn, stimulate vasoconstriction. This vascular contraction elevates calcium concentration in myocardial cells through calcium loading and cyclic adenosine monophosphate formation, enhancing myocardial contractility while reducing myocardial relaxation. On the other hand, an excessive calcium infusion into cardiac cells may cause arrhythmias and, in extreme circumstances, result in abrupt cardiac death (Bers, 2006). Given the slow turnover of myocardial cells, prolonged pressure or volume overload exerts continual stress on these cells. Over time, this stress contributes to myocardial cell apoptosis and necrosis, ultimately resulting in a decline in ventricular contractile function and the onset of HF (Kubo et al., 2008).

However, the remodeling process in HF is a gradual phenomenon, characterized by progressive changes in the mass, composition, and volume of ventricles, ultimately resulting in detrimental effects. As the ventricles continue to expand and myocardial hypertrophy ensues, there is an increase in wall tension, leading to fibrosis and ultimately impairing the heart's contractile function. Additionally, this prolonged remodeling process enhances the apoptosis of myocardial cells. These alterations interact synergistically, exacerbating the progression of HF. The deposition of collagen fibers during HF diminishes myocardial compliance, resulting in dysfunction in ventricular contraction or relaxation (Sabbah et al., 1995). The myocardial interstitium serves as the fundamental framework of myocardial cells, playing essential roles in conducting electrical activity, overseeing the contractile function of myocardial cells, and regulating the migration, differentiation, and proliferation of fibroblasts, which are crucial for maintaining the contraction and relaxation of myocardial cells (Weber, 1989). In the event of HF-induced myocardial injury, cardiac fibroblasts undergo transformation into myofibroblasts, releasing a substantial amount of collagen (Kong et

al., 2014, Murphy et al., 2015). The principal mediator for collagen deposition is believed to be transforming growth factor-β1 (TGF-β1) (Frangogiannis, 2019). TGF-β1 inhibits matrix degradation, promotes extracellular matrix enlargement, and facilitates cardiac fibroblasts transduction (Desmoulière et al., 1993). Under normal conditions, TGF-β1 remains in an inactive state within the extracellular matrix (Frangogiannis et al., 1998, Rifkin et al., 1999). However, during HF, reactive oxygen species (ROS) and acidic environments expedite the release of TGF-β1 (Barcellos-Hoff et al., 1994, Lyons et al., 1988). Furthermore, cardiac fibrosis predominantly expands in the boundary zone of myocardial injury, secreting structural proteins such as collagen I (Col I) and collagen III (Col III) (Cleutjens et al., 1995, Weber, 1989).

2.2.3 Inflammation and Oxidative Stress

An excess of reactive oxygen species relative to antioxidant defenses is known as oxidative stress, and it is one of the primary mechanisms in the pathophysiology of cardiac remodeling and HF. In a failing heart, chronic increase in mitochondrial ROS production can cause mitochondrial DNA (mtDNA) damage, resulting in impaired myocardial cell function (Ide et al., 2001). Moreover, ROS triggers the activation of various hypertrophic signaling kinases and transcription factors, induces cell apoptosis, promotes cardiac fibroblast proliferation, and activates matrix metalloproteinases, leading to the remodeling of the extracellular matrix.

In the pathophysiology of HF, numerous inflammatory cytokines are involved, primarily including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1 (IL-1) (Sharma et al., 2000). The cascade triggered by these inflammatory factors, in conjunction with neurohormonal activation and oxidative stress, can result in endothelial dysfunction and left ventricular dysfunction, contributing to the clinical manifestations of HF.

Under normal circumstances, the heart does not produce TNF-α. TNF-α is only generated in response to acute injury, leading to a significant increase in its concentration. Due to the induction of oxidative stress, TNF-α exerts direct toxicity to myocardial cells, playing a crucial role in the development of left ventricular dysfunction (Rathi et al., 2002). Increased levels of TNF-α in plasma have been found to be an independent predictor of death in individuals with HF and can be used as a gauge for the severity of the condition (Savic-Radojevic et al., 2013).

The study also revealed that IL-1β is an important factor in promoting myocardial remodeling. IL-1 consists of two types of ligands, IL-1α and IL-1β. When myocardial injury occurs, myocardial cells and fibroblasts release IL-1, playing a role in the process of myocardial cell hypertrophy and apoptosis (Hanna and Frangogiannis, 2020). IL-1 can also synergistically inhibit myocardial function when interacting with TNF-α. The level of IL-6 is positively correlated with cardiac function grading and negatively correlated with LVEF. It can induce myocardial cell hypertrophy through the STAT3 transcription factor signaling pathway. IL-6 has predictive value for the prognosis of HF. In the early stages of HF, IL-6 levels first increase, serving as a sensitive indicator (Ridker and Rane, 2021). On the other hand, TNF-α, IL-1, and IL-6 production in HF is downregulated by the anti-inflammatory cytokine IL-10 (Smallie et al., 2010).

2.3 Current Treatments and Recent Innovations

At present, the treatment for HF includes drug therapy and surgical interventions. Common medications include neurohormone antagonists, which can alleviate patients' symptoms. For some patients, it is necessary to implant cardiac resynchronization therapy devices or defibrillators, or even undergo heart transplantation. New

medications and therapeutic approaches, like stem cell therapy, have been researched recently in an effort to better enhance the quality of life for HF patients (Table 2.1).

Table 2.1 A brief summary of HF treatment.

Treatment Method	Effectiveness	Advantages	Disadvantages	Ref.
Medications				
ACEI (e.g., Enalapril)	ACEI inhibits ACE (angiotensin-converting enzyme), significantly lowering blood pressure, improving heart function, and reducing hospitalizations in HF patients, especially those with left ventricular systolic dysfunction.	- Significantly reduces mortality and hospitalization rates in HF patients. - Improves ventricular remodeling and slows disease progression. - Improves long-term prognosis.	- May cause hypotension, hyperkalemia, dry cough, etc. - Contraindicated in patients with severe renal dysfunction or hypotension. - Cannot fully block RAAS, as non-ACE dependent pathways still produce angiotensin II.	(Fröhlich et al., 2018, Svanström et al., 2015)
ARBs (e.g., Valsartan)	ARBs are effective in patients who cannot tolerate ACEI, providing similar benefits in improving HF symptoms.	- More suitable for patients intolerant to ACEI. - Fewer side effects like cough. - Similar efficacy to ACEI in improving HF symptoms and reducing hospitalizations.	- Caution is needed in patients with impaired renal function. - Evidence supporting benefits for all HF patients, especially those without left ventricular dysfunction, is less robust than for ACEI. - Can cause hyperkalemia and hypotension.	(Markan et al., 2019, Pozzi et al., 2023, Kario, 2018)
β-blockers (e.g., Metoprolol)	β-blockers reduce heart rate and load, improving cardiac pumping function and reducing mortality in HF patients, particularly in early stages of HF.	- Significantly reduces mortality and hospitalization rates. - Improves cardiac function and exercise tolerance. - Suitable for most HF patients, especially those with tachycardia.	- May cause bradycardia, hypotension, fatigue, etc. - Caution is needed in elderly patients or those with severe bronchial conditions. - Not suitable for patients with acute decompensated heart failure; should be adjusted based on clinical status.	(Foody et al., 2002, Gheorghiade et al., 2003)
Devices & Surgical Interventions				
Implantable Cardioverter-Defibrillators (ICD)	Reduces cardiac mortality in high-risk patients.	- Effective in preventing sudden cardiac death. - Less invasive than open surgeries.	- Does not treat underlying HF. - Risk of complications (infection, inappropriate shocks).	(Vehmeijer et al., 2016, Elming et al., 2017)

Cardiac Resynchronization Therapy (CRT)	Improves heart function, quality of life, and exercise capacity in specific HF patients (especially with wide QRS complexes).	- Widely used and proven to reduce symptoms. - Non-invasive compared to surgery.	- Does not cure HF or regenerate damaged tissue.	(Ojo et al., 2017)
Left Ventricular Assist Devices (LVAD)	Effective in improving survival and quality of life for end-stage HF patients.	- Life-saving for patients awaiting heart transplants. - Improves prognosis and reduces hospitalizations.	- High risk of complications (e.g., infection, device malfunction). - Requires lifelong management.	(Rogers et al., 2017, Jakovljevic et al., 2017)
Heart Transplant	Considered the gold standard for end-stage HF, with improved survival and quality of life.	- Provides long-term solution for eligible patients. - Significant survival benefit for severe cases.	- Limited by donor organ availability. - Risk of rejection, infection, complications. - Expensive and requires immunosuppression.	(Sanchez-Enrique et al., 2017, Bounader and Flécher, 2024)
Recent Innovations				
SGLT2 Inhibitors (Dapagliflozin)	Reduces the risk of worsening HF and cardiovascular death; improves renal function.	- Safe for patients with low blood pressure. - No adverse effects related to neurohormonal antagonists. - Long-term renal benefits.	- Long-term safety data still evolving. - Limited impact on non-diabetic HF patients.	(Nassif et al., 2021, Solomon et al., 2022)
Viliximab (sGC Stimulator)	Reduces cardiovascular death or HF hospitalization in high-risk patients.	- Anti-inflammatory and antifibrotic effects. - Fewer adverse effects than older treatments.	- Still in early clinical trials. - Limited availability.	(Murphy et al., 2020)
Omecamtiv Mecarbil (Cardiac Myosin Agonist)	Improves heart function parameters like LVEF, but does not significantly reduce mortality or HF hospitalizations.	- Increases myocardial contractility. - Safe without major adverse effects.	- No impact on mortality or long-term clinical outcomes.	(Bernier and Buckley, 2021, Liu et al., 2016)
-Stem Cell Therapy	Demonstrated potential to regenerate heart tissue and improve cardiac function in preclinical and clinical studies.	- Can promote tissue regeneration, angiogenesis, and autocrine/paracrine effects. - Potential for long-term cardiac repair.	- Limited by technical challenges (e.g., stem cell survival, immune rejection). - Needs more research to validate efficacy.	(Nguyen et al., 2016, Nair and Gongora, 2020)

2.3.1 Medications

Neurohormone antagonists, including angiotensin-converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARBs), mineralocorticoid receptor antagonists, and β-blockers, are the primary drugs used to treat HF and can improve its clinical course (Komajda et al., 2018, Komajda et al., 2017, Savarese et al., 2021).

When individuals with left ventricular systolic dysfunction, whether symptomatic or asymptomatic, the rate of hospitalization for HF is considerably decreased by ACEI. Enalapril, bisoprolol, and carvedilol have all been studied in large numbers of patients with severe HF and markedly reduced left ventricular ejection fraction to assess their therapeutic efficacy (1987, 1999, Packer et al., 2001, Pitt et al., 1999). These findings show that medications significantly improve HF patients' symptoms by improving blood circulation, reducing cardiac workload, controlling blood pressure, and regulating heart rate. They also significantly reduce the hospitalization and mortality rates associated with HF (1987, 1999, Packer et al., 2001, Pitt et al., 1999). Despite the use of ACEI, the blockade of the RAAS remains incomplete, with evidence suggesting that non-ACE dependent pathways continue to produce angiotensin II (Ferrario and Mullick, 2017). ARBs have been studied in medium-to large-scale clinical study to assess their safety and possible advantages for patients with systolic function impairment (Wong, 2013). For patients with impaired left ventricular systolic function and HF, combining ARBs with recommended HF treatments (including ACEI) can be considered to improve treatment efficacy.

It is worth noting that patients with severe comorbidities such as hypotension, low cardiac output, and severe renal dysfunction usually cannot tolerate neurohormone modulators. Research has confirmed that due to the hemodynamic limitations of ACEI, patients with severe HF have a mortality rate of over 50% within one year (Kittleson et

al., 2003). Additionally, many studies have proved the feasibility, and safety of valsartan in advanced HF stages and found that the benefits of valsartan for severe patients are still uncertain (Mann et al., 2020). Moreover, patients with advanced HF may have intolerance to valsartan, which could contribute to the worsening of their condition.

Research indicates that valsartan is equally effective as captopril in HF patients and can serve as alternative treatment for those intolerant to ACEI (Demers et al., 2005). However, the combination therapy of valsartan and captopril did not have significant survival benefits compared to using captopril alone. Nevertheless, ACEI remains the preferred treatment for HF patients, while ARBs can be used for those who are significantly intolerant to ACEI. ACEI may be appealing to individuals with HF and those who can preserve left ventricular systolic function, however, there isn't any proof of this at this time from extensive clinical trials.

2.3.2 Devices and Surgical Interventions

Surgical treatment for HF is mainly applicable to patients who have poor responses to medication or more severe conditions. Common surgical treatments include implantable cardioverter-defibrillators (ICD), cardiac resynchronization therapy (CRT), left ventricular assist devices (LVAD), and heart transplantation (Figure 2.2). These treatments can effectively improve heart function, alleviate symptoms, and to some extent, enhance survival rates and quality of life.

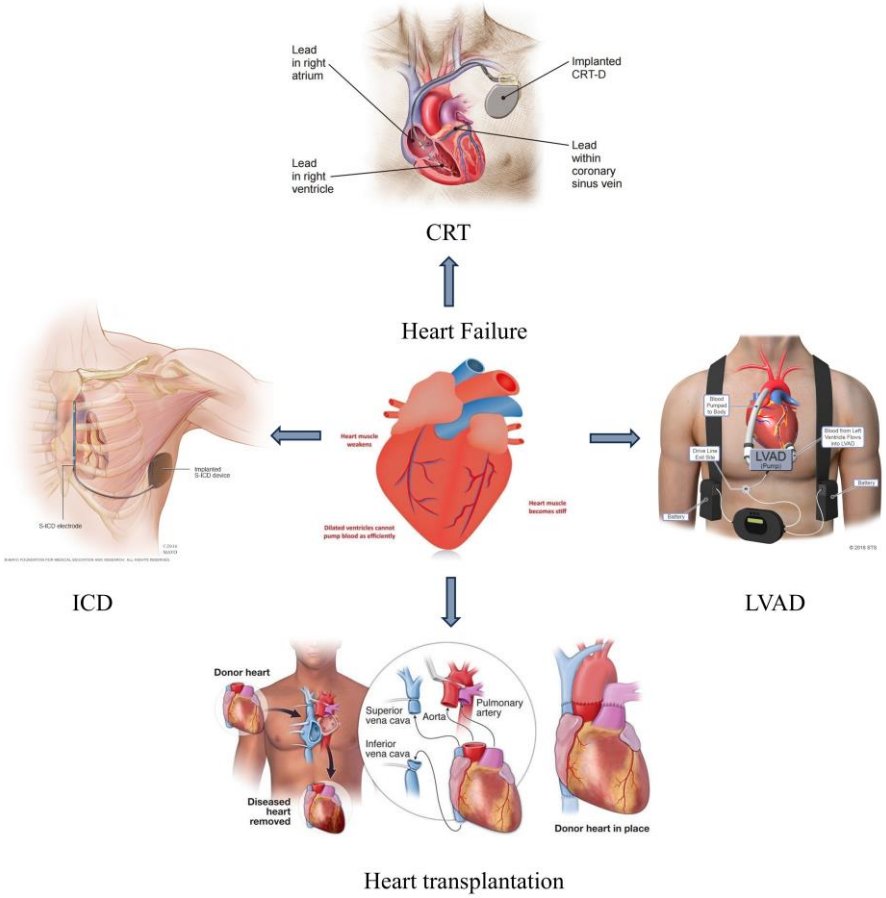

Figure 2.2 Devices and surgical interventions for HF treatment.
For patients with HF who do not respond to medication or have severe conditions, treatment options include LVAD, ICD, CRT, and heart transplantation.

2.3.2(a) Implantable Cardioverter-defibrillators

For individuals who are at a high risk of experiencing cardiac arrest or sudden cardiac death, an implanted cardioverter-defibrillator, or ICD, is an essential therapy option. ICD is a device that can monitor cardiac rhythm and provide electric shocks when necessary to restore normal heart rhythm. ICDs primarily come in two types: transvenous implantable cardiodefibrillators (TV-ICD) and subcutaneous implantable defibrillators (S-ICD). For HF patients with structural heart disease, implementing primary and secondary prevention with ICDs requires optimizing device settings and employing measures such as drug therapy and ablation. These steps help reduce inappropriate ICD discharges and improve efficacy. Numerous studies have confirmed that ICDs are significantly effective and efficient in reducing cardiac mortality

(Begisbayev et al., 2022). Moreover, the economic benefits of ICD treatment have been validated across various healthcare systems (Neyt et al., 2008).

2.3.2(b) Cardiac Resynchronization Therapy

The treatment method for HF patients, CRT, has become the norm. CRT improves the pumping function of the heart by using pacemakers to coordinate the contractions on both sides of the heart. This treatment method is particularly suitable for heart failure caused by asynchronous cardiac contractions. A lot of clinical studies have evaluated the safety and efficacy of CRT, and it has been widely implemented in clinical practice, resulting in higher surgical success rates and reduced postoperative complications (Boriani et al., 2016). In a clinical study, CRT was demonstrated to improve exercise capacity, alleviate HF symptoms, and significantly improve the quality of life for patients with moderate to severe HF (Antoniadis et al., 2017). Additionally, CRT is especially beneficial for HF patients with a wider QRS (Quality, rest, and strength) complex (Cleland et al., 2005). However, while CRT is an beneficial treatment for managing HF, it does not cure the condition or repair damaged myocardial tissue.

2.3.2(c) Left Ventricular Assist Devices

The LVAD is durable and safe treatment method that has been successfully implanted in over 22,000 HF patients (Slaughter et al., 2009). LVAD is a mechanical pump that helps pump blood out of the LV in HF patients. LVADs are suitable for end-stage HF patients who have not responded to drug therapy. Originally intended to serve as a stopgap measure until heart transplantation, LVADs are now more frequently used to treat HF due to improvements in patient prognosis brought about by technological and medical advancements. In a clinical study, Mehra et al. recruited 2,200 LVAD implant patients, and the results showed a reduction in adverse event burden and

hospitalization rates. These advantageous results have been acknowledged in the clinical management of severe HF patients. However, these patients still experienced a high proportion of adverse events related to LVAD (Han et al., 2018).

2.3.2(d) Heart Transplant

Heart transplantation is an effective treatment method to enhance living standards and survival rate of refractory HF patients and is considered a gold standard for treating this condition. Patients with grade III and IV cardiac function who are not improving with medicine and surgery can benefit from heart transplantation (Costanzo et al., 1995). Dilated cardiomyopathy is the main reason for heart transplantation even though ischemic cardiomyopathy is the most common cause of HF because of its lower incidence of complications than ischemic cardiomyopathy (Lund et al., 2013). Right ventricular dysfunction, rejection, infection, and primary graft failure are among the early problems following a heart transplant. Tumor growth and cardiac allograft vasculopathy are examples of late consequences. By thoroughly screening for indications and contraindications and using clear immunosuppressive regimens to diagnose and treat rejection, the results of heart transplantation can be quite beneficial. However, the widespread implementation of heart transplantation is limited by the increased use of mechanical circulatory support devices, donor shortages, and funding constraints. Despite these challenges, there is significant potential to improve the quantity and results of heart transplants by increasing the number of suitable donors and utilizing mechanical circulatory support devices. These advancements could beneficially affect patient prognosis (Bocchi and Fiorelli, 2001).

2.3.3 Recent Innovations

2.3.3(a) New Medications

Recent studies have shown that new drugs, including SGLT2 inhibitors, Viliximab, and omecamtiv mecarbil, have significant benefits for patients with HF. These drugs do not significantly reduce systolic blood pressure and have long-term benefits for renal function, particularly SGLT2 inhibitors. As a result, patients may have a higher tolerance for these treatments (Ameri et al., 2021).

2.3.3(a)(i) Sodium-glucose Cotransporter 2 Inhibitors

Sodium-glucose cotransporter 2 inhibitors (SGLT2 inhibitors) may have an effect on myocardial metabolism, fibrosis, and vascular function in addition to their diuretic and hemodynamic effects (Packer et al., 2017). In the clinical study, dapagliflozin not only improved patients' physical function but also reduced the risk of deterioration for HF patients (McMurray et al., 2019). Regardless of the existence of diabetes, HF patients who received dapagliflozin had an 18% reduced risk of their condition getting worse or dying from cardiovascular causes than those who received a placebo (Kim et al., 2023). Notably, even in patients with baseline systolic blood pressure below 110 mmHg, dapagliflozin is well tolerated, offering new prospects for patients with advanced HF (Serenelli et al., 2020). Most importantly, SGLT2 inhibitors do not have adverse reactions associated with the use of neurohormonal antagonists (such as hypotension, bradycardia, and hyperkalemia), which is a significant advantage for SGLT2 inhibitors in managing fragile patients with advanced HF.

2.3.3(a)(ii) Novel Soluble Guanylate Cyclase Stimulant

Viliximab is a novel soluble guanylate cyclase (sGC) stimulant (Armstrong et al., 2018). The biological messenger nitric oxide (NO) can activate the sGC-cyclic guanosine monophosphate (cGMP) signaling pathway, which is present in the cell

membrane and cytoplasm. This NO-sGC-cGMP signaling pathway plays a crucial role in vasodilation and antiplatelet aggregation within the cardiovascular system. Viliximab exhibits anti-inflammatory and antifibrotic effects, with hypotension being its main adverse reaction. In a randomized and double-blind clinical study involving high-risk patients with chronic HF, those receiving vinzimab had a 10% reduced incidence of cardiovascular death or hospitalization due to HF than those receiving a placebo (Murphy et al., 2020). In addition, compared to the control group, the incidence of adverse events such as symptomatic hypotension and syncope was significantly lower in the viliximab group (Murphy et al., 2020).

2.3.3(a)(iii) Cardiac Myosin Agonist

The new positive inotropic drug, cardiac myosin agonist-Omecamtiv Mecarbil (OM), also named CK-1827452, is a highly selective small molecule compound. OM increases myocardial contractility by binding to the heavy chain of myosin and activating the S1 domain, without altering the concentrations of cyclic adenosine monophosphate and Ca^{2+} in myocardial cells (Cleland et al., 2011). An analysis comprising six randomized controlled studies (RCTs) with 9,429 participants evaluated the safety and effectiveness of OM for HF (Xiong et al., 2015). The evidence shows that OM can significantly improve cardiac function parameters, including clinical evaluation score, LVEF, LVEDV, LVESV, and SV in HF patients, without increasing the occurrence of adverse events. However, OM did not demonstrate significant benefits in terms of cardiovascular disease (CVD) mortality and HF hospitalization composite events, HF hospitalization rate, CVD mortality rate, or all-cause mortality rate in HF patients(Liu et al., 2016).

2.3.3(b) Cell Replacement Therapy

At present, treatments for HF primarily focus on alleviating symptoms and prolonging patients' lifespans. With the massive loss of myocardium in HF therapy that could achieve heart tissue restoration is scarce. Stem cells are the only cell source that can proliferate indefinitely and develop into particular types of adult cells with their capacity for self-renewal and differentiation (Yamanaka, 2020). Mesenchymal stem cells from bone marrow, cardiac stem cells, embryonic stem cells (ESC), and induced pluripotent stem cells (iPSCs) are examples of stem cells (Figure 2.3). Numerous preclinical studies have confirmed that stem cells can enhance cardiac function and mitigate the remodeling of the LV in heart disease (Poglajen et al., 2020). Research has shown that these stem cells can promote angiogenesis and possess autocrine or paracrine abilities, thereby enhancing cardiac function. In studies involving immunodeficient rats, injecting stem cells into the myocardial injury area resulted in the formation of dynamic myocardial tissue in the central region of the myocardial wall (Bolli et al., 2011). This process promotes myocardial regeneration and angiogenesis, thereby comprehensively improving cardiac function. Similar positive results have also been observed in clinical studies, where autologous stem cells are injected into the hearts of HF patients through coronary arteries (Gupta et al., 2021). The LVEF considerably improved after four months. The research on cell therapy's effects on pathological alterations and cardiac physiology is growing, which is encouraging researchers to focus on cell regeneration.