# CHAPTER 1

# Introduction and Thesis Outline

## GENERAL INTRODUCTION

Acute myocardial infarction (MI) constitutes a major health problem worldwide.[1] Over the last decades, the prognosis after MI has improved due to the introduction of primary percutaneous coronary interventions (PCI) and pharmacologic therapies that prevent reocclusion of the coronary artery and effectively mitigate cardiovascular risk factors.[2] Nevertheless, rates of death and occurrence of heart failure after MI remain substantial.[3] The size of the myocardial scar, i.e. infarct size, is the strongest prognostic determinant of clinical outcomes.[4] Timely and effective reperfusion by PCI is important to rescue the viable myocardium and limit the infarct size. However paradoxically, reperfusion itself might also lead to additional myocardial injury, which can contribute up to 50% of the final infarct size.[5,6] Mechanisms underlying this so called "ischemia-reperfusion (I/R) injury" include rapid pH changes, calcium overload, cardiomyocyte hypercontracture, bursts of reactive oxygen species (ROS) and inflammatory cells, and adverse changes to the coronary microcirculation.[7–9] Effective treatments targeting I/R injury in humans, which may potentially limit infarct size and improve prognosis, are currently lacking. Therefore, the search continues.[10] In this thesis, we investigated two classes of compounds that might provide a therapy to target I/R injury: free thiols and ketone bodies.

### Thiol-based cardioprotection

Thiols consist of a large family of compounds with a sulfhydryl (–SH) group attached.[11] They exist both intracellular (e.g. glutathione, cysteine) and extracellular (e.g. free protein thiols). Thiols are critically involved in redox signaling, but also function as potent antioxidants. Under conditions of oxidative stress, thiols are being oxidized by ROS, leading to the formation of disulfide bonds, which neutralizes ROS and prevents them from inflicting protein and lipid modifications and the subsequent structural cellular damage.[11] Levels of circulating free thiols have been linked with disease severity and outcomes in a variety of oxidative stress-associated diseases.[12] Since oxidative stress is one of the hallmarks of I/R injury, free thiols might be of additional value during MI, especially since free thiols are also amendable to therapeutic modulation. However, to date studies linking free thiols to disease outcomes or investigating their therapeutic potential after MI are lacking.

Hydrogen sulfide ($H_2S$) is considered as the smallest member of the thiol family.[11] $H_2S$ is a gaseous signaling molecule which is present in mammalian cells and serves as a physiological mediator of many tissue processes.[13] Next to its direct ROS scavenging function, $H_2S$ also preserves mitochondrial function and structure, and has vasodilatory, anti-apoptotic, anti-fibrotic, anti-inflammatory, pro-angiogenic and indirect antioxidant properties.[14] In animal studies, disturbance of endogenous $H_2S$ production is associated with development of cardiovascular pathology and increase of infarct size,[15,16] whereas administration of $H_2S$-donating compounds provides cardioprotection.[17,18] $H_2S$-donors include inorganic salts, plant derived $H_2S$-donors and synthetic $H_2S$-donors.[19] Of these, only sodium thiosulfate (STS) is approved for clinical use yet.[20] STS is a strong antioxidant, anti-inflammatory and vasoactive compound, that is also able to release $H_2S$, especially under ischemic conditions.[21] For many

years, STS is used to treat cyanide poisoning, calciphylaxis and more recently also cisplatin-related side effects, including ototoxicity and kidney damage.[22–25] A wealth of preclinical evidence demonstrates infarct-sparing effects for $H_2S$/STS.[18] However, to date, no translational attempt was performed in humans.

**Ketone bodies**

Ketone bodies (KBs) are endogenous metabolites that are produced by the liver, especially under conditions of insulin deprivation, prolonged fasting, extreme exercise and increased sympathetic nervous system activation, a situation which occurs during cardiovascular disease.[26] Under physiological circumstances, the heart predominantly uses glucose and fatty acids as metabolic substrates and KBs are only responsible for <10% of the ATP production.[26] KBs can be used by the heart as an efficient metabolic substrate, since KBs require less oxygen per molecule of ATP produced than glucose or fatty acids.[27] Furthermore, other pleiotropic effects of KBs have been demonstrated, including beneficial effects on gene transcription, inflammation and oxidative stress, endothelial function, cardiac remodeling, and cardiovascular risk factors.[28] In 2016 it was demonstrated, that in patients with heart failure the heart reprograms towards ketone metabolism and that in this condition circulating KBs as well as the cardiac uptake of KBs are increased.[29] Since then, preclinical studies describing pleiotropic effects of KBs and clinical studies advocating therapeutic potential of interventions that enhance circulating KB levels are rapidly accumulating, but these are mainly restricted to heart failure.[30–34] The potential role of KBs in I/R injury, specifically in patients presenting with ST-segment elevation MI (STEMI), remains unknown. Unraveling the role of KBs during I/R injury may support the development of novel therapies for STEMI.

## AIMS AND OUTLINE OF THIS THESIS

The overall aim of this thesis is to explore potential new pharmacological strategies to protect the heart against I/R injury in patients presenting with STEMI. In **Part I**, we aim to substantiate evidence for possible new therapeutic strategies by investigating associations with outcomes after STEMI and heart failure. In **Part II**, we aim to evaluate the safety and tolerability of the $H_2S$- and thiol-donating compound STS, in patients presenting with acute MI and we aim to test its efficacy in reducing myocardial I/R injury.

In **Part I** of this thesis, we describe associations of circulating KBs with functional outcomes after STEMI (**Chapter 2**). In **Chapter 3** and **4** we investigate associations of circulating free thiols with functional outcomes after STEMI and clinical outcomes in a large cohort of patients with new-onset or worsening heart failure, respectively.

In **Part II** of this thesis, we investigate the effects of one specific thiol-donating strategy specifically, the $H_2S$-donor STS, in patients presenting with STEMI. In **Chapter 5**, the safety and tolerability of STS in patients presenting with acute coronary syndrome is studied. **Chapter 6** describes the rationale and design of the Groningen Intervention Study for the Preservation of cardiac function with sodium thiosulfate after STEMI (GIPS-IV), a randomized controlled trial in which the hypothesis is investigated that STS reduces myocardial infarct size. The primary results of this trial are presented in **Chapter 7**. Finally, we discuss the main findings and conclusion of this thesis, as well as the future perspectives, in **Chapter 8**. To summarize, **Figure 1** describes the compounds that will be investigated in this thesis and their potential cardioprotective effects.

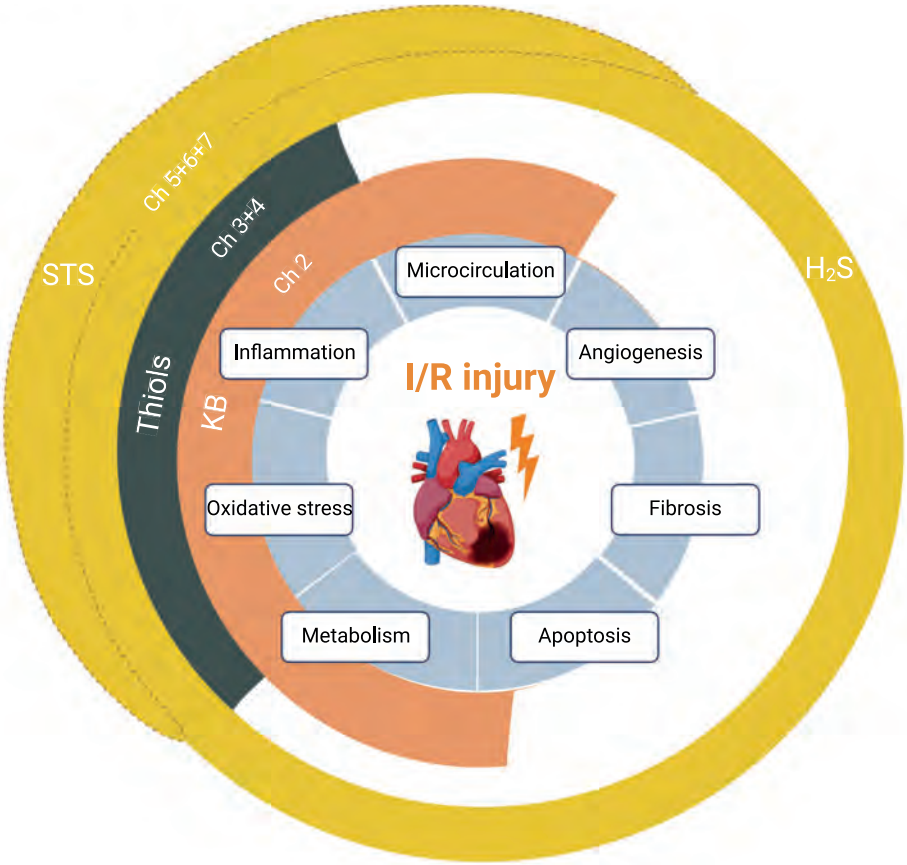


***Figure 1 | Potential cardioprotective mechanisms of compounds that will be investigated in this thesis***

*Abbreviations: Ch, chapter; $H_2S$, hydrogen sulfide; I/R, ischemia-reperfusion; KB, ketone bodies; STS, sodium thiosulfate; Thiols, free thiols.*

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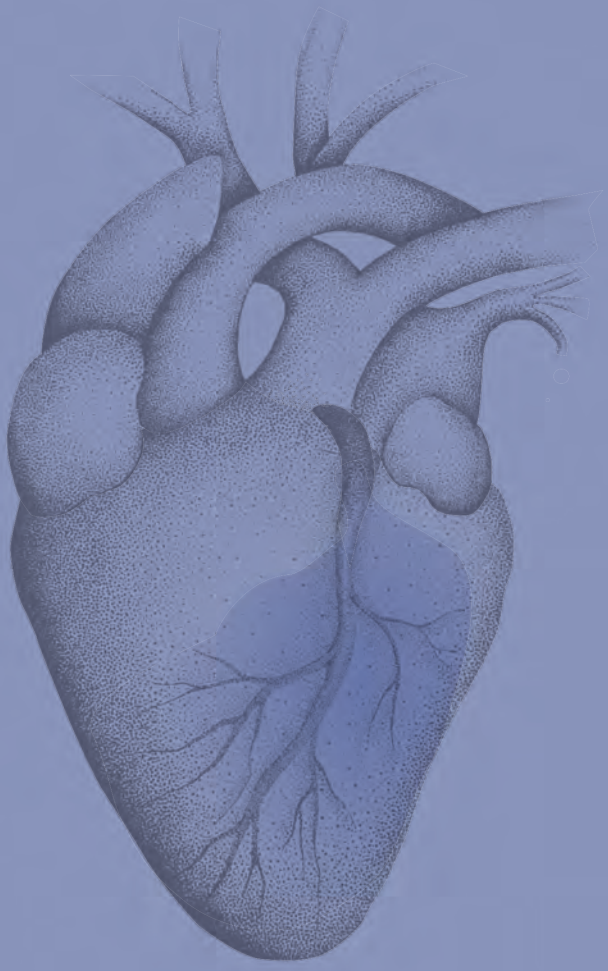

PART I

# Possible new therapeutic targets for ischemia-reperfusion injury