EFFECTS OF HEME OXYGENASE – 1 MODULATION IN MYOCARDIAL INFARCTION IN RATS

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Effects of Heme oxygenase – 1 modulation in Myocardial infarction in rats

Abstract:

Background: Myocardial infarction (MI) has been a major health concern mainly caused by reduced blood flow to the myocardium due to occlusion in the coronary circulation. Although the early reperfusion is vital to salvage the ischemic myocardium, the very reperfusion itself can cause injury to the myocardium. Currently available treatment approaches mostly aim to restore blood flow but they do not adequately address the molecular mechanisms that cause immediate and long term changes in MI. Heme oxygenase-1 (HO-1), a stress-response enzyme, plays an important role to produce cytoprotective effects by catalytic degradation of Heme to biliverdin, carbon monoxide (CO), and free iron.

Objectives: Current study aims to evaluate the cardioprotective effects of Heme oxygenase-1 modulation in MI in rats and its role in activation of downstream components of important pro-survival signaling pathways.

Methods: The current study was conducted in using three methodological approaches: 1) Ex-vivo: IRI was induced in isolated rat heart using Langendorff perfused heart model 2) In vivo: Isoproterenol (ISO)-induced MI in rats; and 3) In vitro: ISO-induced injury in H9c2 cardiomyoblasts. HO-1 was modulated using HO-1 inducer, Hemin and HO-1 inhibitor, Zinc Protoporphyrin IX. For mechanistic evaluation, specific pharmacological inhibitors of PI3K (Wortmannin), Protein Kinase C (Chelerythrine), Nitric Oxide Synthase (L-NAME), and ATP-sensitive potassium (K_{ATP}) channels (Glibenclamide) were used. Various parameters such as cardiac functional parameters, myocardial injury biomarkers, oxidative stress markers, HO-1, PI3K, and histopathology were assessed.

Results: HO-1 induction by Hemin exerted dose-dependent cardioprotection as evident through improvement in cardiac function and electrophysiological stability; reduction in the level of myocardial injury markers; attenuation of oxidative stress and preservation of myocardial architecture. HO-1 inhibition resulted in the abolished aforementioned protective changes. Mechanistic evaluation through the inhibition of PI3K, PKC, NOS, and K_{ATP} pathways revealed involvement of multiple pro-survival mechanisms, with significant involvement of PI3K signaling.

Conclusion: HO-1 induction by Hemin exerts cardioprotection against myocardial injury. The protective effect is mediated through the activation of multiple pro-survival signaling mechanisms with dominant involvement of PI3K signaling.

Brief Description of the State of the Art on the Research Topic

Introduction to Myocardial Infarction

Cardiovascular diseases, especially ischemic heart disease (IHD), are the chief contributors of mortality and morbidity worldwide [1]. Myocardial infarction, being the most severe consequence of coronary artery diseases, results from the death of the cardiac myocytes due to ischemia caused by occlusion in the coronary artery mainly due to the formation of atheromatous plaque [2]. MI results in immediate and long term changes in the myocardium at cellular and molecular level irreversibly, leading to cardiac myocyte death. [3]. Although the early reperfusion is vital to salvage the ischemic myocardium, the very reperfusion itself can cause injury to the myocardium, referred to as ischemia-reperfusion injury (IRI). IRI results from rapid increase in reactive oxygen species (ROS) and pro-inflammatory cytokines as well as calcium overload, which triggers various cell death pathways. Over the time, these pathological changes culminate into adverse cardiac complications and death [4]. Conventional treatment and management strategies primarily focus on restoring blood flow and maintaining cardiac function [5]. However, these strategies do not adequately address underlying cellular and molecular mechanisms that cause irreversible changes and complications in the myocardium. Therefore, cardioprotection requires multi-target approaches to withstand stressful events and enhance survival [6].

Heme Oxygenase-1 (HO-1): An Endogenous Defence Mechanism

Heme oxygenase-1 (HO-1) catalyses the breakdown of heme into biliverdin (BV) which is subsequently converted to bilirubin (BR) by the action of an enzyme biliverdin reductase (BVR), carbon monoxide (CO), and ferrous iron (Fe^{2+}) [7]. HO-1 is physiologically induced by cells during stressful events. There are various beneficial roles of HO-1 and catalytic breakdown products of heme such as include as immunomodulation, anti-inflammatory, and cytoprotective effects. These cytoprotective effects makes HO-1 a suitable candidate to exert protection during pathological changes during of cardiovascular diseases, especially MI and its complications [8]

There are various inducers of HO-1 such as ultraviolet radiation, hypoxia, heavy metals, hydrogen peroxide, and nitric oxide (NO). They induce HO-1 by activating its regulatory mechanisms such as transcription factors nuclear factor kappa B (NF-κB), nuclear factor erythroid 2-related factor 2 (Nrf2), and activator protein-1 (AP-1) [9]. Hemin also induces HO-1 by activating Nrf2 signaling and thereby promoting cytoprotection [10]. Zinc Protoporphyrin

IX (ZnPP IX) is a known inhibitor of HO-1. Hemin and ZnPP IX are frequently used for the evaluation of functional role of HO-1 and its mechanistic role [11].

Various signalling pathways are involved in the pathogenesis and survival of cardiac myocytes. They regulate various processes at cellular and molecular level such as inflammation, oxidative stress, apoptosis, myocardial fibrosis, and angiogenesis. All these changes are of important targets in the pathogenesis of MI and associated complications [12]. HO-1, having a potential to activate prosurvival mechanisms and signaling pathways, could be a promising approach to exert cardioprotection during the pathogenesis of MI. The current study was designed to evaluate the cardioprotective effects of HO-1 induction by Hemin and to find of its role in the activation of pro-survival signaling pathways, thereby offering mechanistic insights.

Definition of the Problem

Myocardial infarction (MI), a serious consequence of ischemic heart diseases, is one of the life-threatening healthcare emergencies worldwide. Current treatment approaches such as thrombolytic therapy, percutaneous coronary intervention (PCI), β-blockers, ACE inhibitors, and antiplatelet agents have significantly improved primary survival. However, they have not been able to prevent ischemia-reperfusion injury (IRI) and its associated consequences that culminates into contractile dysfunction and heart failure [13].

These standard-of-care interventions primarily focus on restoring perfusion but they neither modulate cellular responses to oxidative or inflammatory stress nor address the molecular mechanisms underlying cardiomyocyte death, mitochondrial dysfunction, redox imbalance, or long-term remodelling. As a result, there is an urgent requirement to for adjunctive therapies that target the intracellular signaling mechanisms associated with death and survival of cardiac myocytes during myocardial infarction and its complications.

Heme Oxygenase-1 (HO-1) is a highly conserved, stress-inducible enzyme that exerts antioxidant, anti-inflammatory, anti-apoptotic, and cytoprotective effects through the degradation of pro-oxidant heme into biliverdin, bilirubin, carbon monoxide (CO), and free iron. These metabolites have been shown to protect against tissue damage in hepatic, renal, pulmonary, and neurological injury models [14].

HO-1 is well studied for its cytoprotective effects. However, HO-1’s protective role in the myocardial infarction and associated complications, especially its potential to modulate prosurvival signaling pathways, has been inadequately explored.

Multiple cellular and molecular pathways govern survival of myocardium during IRI. Targeting these prosurvival pathways may reduce IRI associated damage and may exert cardioprotection [15]. Several studies have reported PI3K, PKC, NOS, and KATP channels as important prosurvival signaling during myocardial IRI. PI3K signaling preserves the myocardium during ischemia reperfusion injury by halting the process of apoptosis, reducing oxidative stress, suppressing inflammation, and promoting angiogenesis and tissue repair [16]. PKC exert cardioprotection in myocardial ischemia/reperfusion injury by regulating redox signaling, calcium homeostasis, cell death, oxidative stress, and mitochondrial function [17]. Nitric oxide produces vasodilation, anti-inflammatory actions, and mitochondrial stabilization and thus exert protective effect during IRI [18]. ATP-sensitive potassium channels protect the heart during IRI by preventing calcium overload, reducing oxidative stress, conserving energy, protecting mitochondrial function, improving blood flow, and modulating inflammation [19]. HO-1 may exert cardioprotection by activating one or more aforementioned pro-survival signaling mechanisms during MI and associated changes.

Current study aims to evaluate cardioprotective effects of HO-1 modulation by Hemin (inducer) and Zinc Protoporphyrin IX (inhibitor). The study also investigates the potential of HO-1 to activate prosurvival mechanisms by using pathway-specific inhibitors of PI3K, PKC, NOS, and K_{ATP} to delineate their roles in survival of myocardium across ex vivo, in vivo, and in vitro models. This would reveal new therapeutic targets beyond conventional reperfusion strategies, thereby facilitating the development of novel adjunctive cardioprotective approaches. This would also lay groundwork for clinical translation of HO-1 modulation in ischemic heart disease.

Objectives and Scope of the Work

Primary objective:

- The primary objective of current research is to evaluate the effects of Heme Oxygenase-1 (HO-1) modulation in myocardial infarction (MI) and to examine its potential interaction with key pro-survival signaling pathways using ex vivo, in vivo, and in vitro experimental models.

Specific Objectives:

- To evaluate the effects of HO-1 modulation in myocardial ischemia–reperfusion injury using isolated rat heart preparations (ex vivo).

- To evaluate the effects of HO-1 modulation in isoproterenol-induced myocardial infarction in rats (in vivo).
- To evaluate the effects of HO-1 induction against isoproterenol-induced myocardial injury in H9c2 cardiomyoblasts (in vitro).
- To assess the changes in cardiac functional, biochemical, and histopathological parameters following HO-1 modulation across ex vivo, in vivo and in vitro models.
- To study the effects of HO-1 induction and inhibition using Hemin and Zinc Protoporphyrin IX (ZnPP IX), respectively.
- To investigate the potential role of prosurvival signaling pathways such as PI3K, PKC, NOS, and K_{ATP}, in HO-1-mediated effects using pathway-specific inhibitors.

Scope of the Work

The study is confined to the evaluation of effects of HO-1 modulation in ex vivo, in vivo and in vitro experimental models of myocardial injury and does not extend to clinical evaluation. The pharmacological modulation of HO-1 is restricted to the use of Hemin as HO-1 inducer and Zinc Protoporphyrin IX (ZnPP IX) as an HO-1 inhibitor across various experimental models. he mechanistic investigations are limited to the specific pro-survival signaling pathways such as PI3K, PKC, NOS, and K_{ATP} channels. The interpretation of findings is limited to the assessment of various parameters indicating myocardial injury or survival, including cardiodynamic indices (LVDP, LVEDP, dp/dt_{max}, dp/dt_{min}, BPM, and coronary flow), biochemical markers of cardiac injury (CK-MB, LDH), oxidative stress markers (MDA, SOD, Catalase), and HO-1 and PI3K activity.

Original contribution by the thesis

Current study contributed to the field of cardiovascular pharmacology by extending current understanding of biological importance of Heme oxygenase-1 (HO-1). The study specifically focuses on elucidating the role of key prosurvival signaling mechanisms activated following HO-1 induction.

The key contributions of this work are outlined below:

- Protective role of HO-1 in the myocardial injury was established trough the improvement in functional, biochemical and histopathological parameters following HO-1 induction across in vivo, ex vivo and in vitro experimental models. HO-1 inhibition by Zinc protoporphyrin IX attenuated theses protective effects, confirming the protective role.

- Significant contribution related to the mechanistic insights about HO-1 mediated cardioprotection was also provided through investigation of potential involvement of prosurvival signaling pathways such as PI3K, PKC, NOS, and K_{ATP} following HO-1 induction. Significant role PI3K signaling in the HO-1 mediated cardioprotection among the investigated pathways was also established through the findings that inhibition of this pathway results in maximum attenuation of the protective effects.
- Overall, this study advances the current understanding of protective effects of HO-1 by providing mechanical insights through the investigation of prosurvival signaling pathways involved in cardioprotection following HO-1 induction. Based on these observations, future experimental investigations and therapeutic strategies targeting HO-1 could be devised.

Methodology of research:

Drugs, chemicals and reagents

Hemin, Chelerythrine and Zinc protoporphyrin IX were obtained from Sigma-Aldrich. Isoproterenol (ISO), L-NAME (N(ω)-nitro-L-arginine methyl ester) and Wortmannin were procured from Tokyo Chemical Industry India Pvt. Ltd. Biochemical kits for CK-MB (creatine kinase-myocardial band) and LDH (lactate dehydrogenase) were acquired from ARKRAY, Inc. An ELISA (enzyme-linked immunosorbent assay) kit for HO-1 was sourced from Bioassay Technology Laboratory, Shanghai Korain Biotech Co., Ltd. PI3K ELISA kit was received from Allianz Biosciences Pvt Ltd.

Animals

Male Wistar albino rats weighing between 250-300 g were procured from Jai Research Foundation. Animals were maintained and all experimental procedures were performed following the established guidelines set by CCSEA (Committee for Control and Supervision of Experiments on Animals) and the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. The Institutional Animal Ethics Committee (IAEC) reviewed and approved the experimental protocol under approval number RPCP/IAEC/2020-2021/R16.

Ex-vivo study: IRI in Isolated perfused rat heart preparation

The study employed an isolated rat heart model using the Langendorff perfusion technique for the ex vivo experiment [20]. Rats were heparinised and anaesthetised intraperitoneally with Ketamine (75 mg/kg) and Xylazine (10 mg/kg). Once deep anaesthesia was confirmed, hearts were excised and promptly transferred into in chilled Krebs–Henseleit (K-H) buffer adjusted

to pH 7.4. Cannulation of the hearts was performed through the aorta and mounted onto a Langendorff apparatus. The hearts were retrogradely perfused with Krebs–Henseleit buffer at a constant flow rate of 15ml/min, maintained at 37°C, and continuously aerated with carbogen (95% O_2 and 5% CO_2 mixture). A fluid-filled latex balloon, connected to a pressure transducer (SS13L), was inserted into the left ventricle to measure cardiac functional parameters. Preload adjusted to 5-10 mm Hg through balloon inflation. The cannulated hearts were allowed to stabilized. Ischemia-reperfusion injury (IRI) was then induced by subjecting the hearts to 30 minutes of ischaemia, followed by 90 minutes of reperfusion.

Experimental protocol

The experiment was conducted in two parts. Animals were divided in nine groups having six rats per group.

Part 1: Dose optimization of Hemin:

The first part of the study was conducted to find out the optimal dose of Hemin for the HO-1 induction and its associated cardioprotection.

- **Group 1:** Isolated hearts were perfused with K-H buffer and, then subjected to the induction of IRI.
- **Group 2:** Isolated hearts were perfused with K-H buffer containing 10 µM Hemin and, then subjected to the induction of IRI.
- **Group 3:** Isolated hearts were perfused with K-H buffer containing 50 µM Hemin and, then subjected to the induction of IRI.
- **Group 4:** Isolated hearts were perfused with K-H buffer containing 100 µM Hemin and, then subjected to the induction of IRI.

Part 2: Mechanistic study using pathway inhibitors:

The second part of the study was conducted to investigate the potential involvement of pro-survival signaling pathways in HO-1 mediated cardioprotection. Pathway-specific inhibitors were used for this purpose.

- **Group 5:** Isolated hearts were perfused with K-H buffer containing 50 µM Zinc protoporphyrin IX (HO-1 inhibitor) and, then subjected to the induction of IRI.
- **Group 6:** Isolated hearts were perfused with K-H buffer containing 10 µM Chelerythrine (PKC inhibitor) and Hemin and, then subjected to the induction of IRI.

- **Group 7:** Hear Isolated hearts were perfused with K-H buffer containing 100 µM L-NAME (NOS inhibitor) and Hemin and, then subjected to the induction of IRI.
- **Group 8:** Isolated hearts were perfused K-H buffer containing 10 nM Wortmannin (PI3K inhibitor) and Hemin and, then subjected to the induction of IRI.
- **Group 9:** Isolated hearts were perfused with K-H buffer containing 10 µM Glibenclamide (K_{ATP} blocker) and Hemin and, then subjected to the induction of IRI.

Evaluation of parameters:

Following the experiment, heart tissues were homogenized, and HO-1 activity was assessed using a commercial ELISA kit as per the manufacturer's protocol. Throughout the experiment, cardiac functional parameters such as LVDP (left ventricular developed pressure), LVEDP (left ventricular end-diastolic pressure), dp/dt_{max} (peak rate of contraction), dp/dt_{min} (peak rate of relaxation), BPM (Beats per minute/heart rate), and coronary flow were recorded using Biopac data acquisition system. Parameters were evaluated before ischemia and after reperfusion. % recovery was calculated as the ratio of post-ischemia-reperfusion (post-I/R) values to the corresponding baseline (pre-ischemic) values, expressed as a percentage [21][22]. Levels of CK-MB and LDH in coronary effluent were quantified before ischaemia and immediately after reperfusion according to the protocols of commercially available kits using biochemical analyser. At the end of the reperfusion period, heart tissue homogenates were prepared and MDA (Malondialdehyde) and SOD (Superoxide dismutase) were assessed to evaluate oxidative stress and antioxidant defence [23][24]. After the experiment, hearts were rinsed with PBS and fixed in 10% formalin for the purpose of cell fixation. The formalin-fixed samples were submitted to an external pathology laboratory for histopathological analysis.

In-vivo study: Isoproterenol-Induced Myocardial Infarction in rats

Experimental Protocol

In the present study, Animals were randomly divided in three groups containing six animals in each group.

- **Normal control group:** Animals were given Dimethyl Sulfoxide (DMSO) through intraperitoneal (IP) route on alternate days for 7 days. Animals also received normal saline through subcutaneous (SC) route for the last two days of the study.
- **Disease control group:** Animals were given DMSO through IP route on alternate days for 7 days. Isoproterenol (ISO) (45 mg/kg) was administered via SC route for the last 2 days of the study.

- **Hemin treated group:** Hemin (4mg/kg, IP) was administered on alternate days for 7 days and ISO (45 mg/kg) was administered via SC route for the last 2 days of the study.

Evaluation of parameters:

Electrocardiogram (ECG) analysis was performed to assess cardiac abnormalities induced by isoproterenol (ISO) using the BIOPAC MP36 System. Key parameters including the ST segment, Q wave and beats per minute (BPM) were evaluated [25]. Cardiac injury biomarkers such as CK-MB and LDH were quantified using commercially available assay kits, following manufacturer's protocols. Heart tissue homogenates were prepared and used to evaluate oxidative stress marker malondialdehyde (MDA) along with anti-oxidant enzyme, including catalase (CAT), and superoxide dismutase (SOD) [26][27]. Homogenate of the hearts from each experimental groups were estimated for the HO-1 activity. For the same, ELISA method was employed [28] according to the protocol of commercial kit provided by manufacturer (Bioassay Technology Laboratory, Shanghai Korain Biotech Co., Ltd.). The levels of PI3K in heart tissue homogenates from each experimental group were quantified using an enzyme-linked immunosorbent assay (ELISA) kit [29] following the protocol provided by Allianz Biosciences Pvt Ltd. After euthanasia, hearts from each group were excised and rinsed with normal saline to eliminate residual blood. The cardiac tissues were the fixed in 10% formalin for preservation. The fixed samples were submitted to a pathology laboratory for histological examination [30].

In-vitro study: ISO-induced myocardial injury in H9c2 cardiomyoblasts

H9c2 cells were purchased from the National Centre For Cell Science, Pune. Cells were cultured in DMEM supplemented with 10% FBS and antibiotics (100U/mL penicillin and 100U/mL streptomycin) at 37∘C in a humidified atmosphere of 5% CO2. When the cells were grown to a density of 80%–90% in culture medium, cell passage was done. The medium was changed 2–3 times per week. The ISO model of H9c2 cells was built to mimic myocardial injury in vivo. The cells were pretreated with Hemin at different concentrations for 2 h and then exposed to isoproterenol (80 μM) for 24 h [31].

Experimental protocol

- **Group 1:** Control
- **Group 2:** Isoproterenol (80 μM)
- **Group 3:** Hemin (10 μM) + Isoproterenol (80 μM)
- **Group 4:** Hemin (30 μM) + Isoproterenol (80 μM)

- **Group 5:** Hemin (60 µM) + Isoproterenol (80 µM)
- **Group 6:** Hemin (90 µM) + Isoproterenol (80 µM)

Evaluation of parameters:

Cell viability was evaluated using MTT assay by measuring absorbance 570 nm using a microplate reader. Level of creatine kinase (CK-MB) in culture supernatant were measured by commercially available kits using biochemical analyser. The estimation of PI3K from cell lysate was performed using enzyme linked immunosorbent assay (ELISA) kit.

Statistical analysis:

Results were expressed as Mean±SEM. Data were analysed by one-way ANOVA followed by Turkey's multiple comparison tests. All analysis was done using GraphPad Prism software. $p<0.05$ was considered to be statistically significant.

Results: Ex-vivo study (IRI in Isolated perfused rat heart preparation)

Part 1: Determination of optimal dose of Hemin for the HO-1 induction and cardioprotection.

HO-1 activity elevated in a dose dependent manner following Hemin treatment. Hearts treated with 10 µM hemin, 50 µM hemin and 100 µM hemin showed significantly elevated HO-1 activity compared to the control group**.** Hemin treatment resulted in significant improvement in cardiac function following ischemia-reperfusion. Significant recovery was observed in LVDP and LVEDP in hearts treated with different doses of hemin when compared to the hearts of untreated group. Similarly, dP/dt_{max} significantly improved in the 100 µM hemin group, and dP/dt_{min} improved significantly in the 50 µM and 100 µM hemin groups**.** BMP and coronary flow also improved in a dose-dependent manner, with significant recovery in the coronary flow (CF) of hearts treated with 100 µM hemin**.** CK-MB and LDH levels were significantly reduced following hemin treatment in a dose-dependent manner. CK-MB was significantly lower in the 50 µM and 100 µM hemin groups, while LDH levels were significantly reduced in all the hemin treated groups compared to control. Hemin treatment mitigated oxidative stress, indicated by reduced MDA levels and enhanced SOD activity in heart tissue homogenates. MDA levels significantly reduced across all hemin treated groups. SOD activity increased significantly in the 10 µM, 50 µM and 100 µM hemin treated groups in a dose-dependent manner. Histopathological analysis revealed that IRI altered the myocardial architecture causing myocardial necrosis, interstitial edema, contraction bands, and fibre separation. These changes

were markedly reduced in hemin treated groups, indicating structural preservation and reduced tissue damage.

Part 2: Evaluation of pro-survival signaling pathways involved in HO-1 mediated cardioprotection

Hearts treated with hemin showed significantly elevated HO-1 activity compared to control group. Treatment with the HO-1 inhibitor ZnPP IX significantly reduced HO-1 activity. Groups treated with hemin and various pathway inhibitors maintained elevated HO-1 activity, confirming that the inhibitors did not directly affect HO-1 induction. Hemin treatment significantly improved dP/dt_{max}, dP/dt_{min}, LVDP, LVEDP and coronary flow compared to control. Although BPM was improved in hemin treated hearts, the difference was not statistically significant. ZnPP IX abolished these improvements, indicating that these effects were mediated through HO-1 activity. Moreover, co-administration of hemin with specific inhibitors of signaling pathways-namely Wortmannin (PI3K inhibitor), Chelerythrine (PKC inhibitor), L-NAME (NOS inhibitor), and Glibenclamide (K_{ATP} inhibitor)-led to either partial or complete attenuation of these functional improvements. For instance, the recovery of LVDP was significantly impaired in the groups treated with PKC an PI3K inhibitors and the recovery of LVEDP was significantly impaired in the groups treated with hearts treated with PKC, NOS an PI3K inhibitors. Similarly, dP/dt_{max} was significantly reduced in the NOS inhibited group, while dP/dt_{min} declined in both NOS and PI3K inhibitor treated hearts**.** BPM did not exhibit a significant change in any of the inhibitor treated groups while, coronary flow was significantly reduced upon inhibition of PKC, NOS, PI3K and K_{ATP} channels, further confirming involvement of these pathways in HO-1 mediated cardioprotection. Levels of LDH as well as CK-MB, which were significantly reduced by hemin, increased significantly upon co-treatment with ZnPP IX or pathway inhibitors, suggesting that the cardioprotective effects of HO-1 involve these signaling pathways This trend was observed across all groups treated with pathway inhibitors. MDA levels, which were lowered by hemin, increased significantly with ZnPP IX and other pathway inhibitors, indicating enhanced lipid peroxidation due to oxidative stress. SOD activity, which was increased with hemin treatment, decreased significantly in ZnPP IX and inhibitor treated groups, reflecting reduced antioxidant defence. Histological analysis revealed minimal tissue injury in Hemin-treated group, characterized by preserved myocardial architecture and reduced signs pathological changes. In contrast, hearts treated with ZnPP IX or pathway-specific inhibitors exhibited features of IRI such as myocardial necrosis, interstitial edema, contraction band and fibre separation at varying degree. These histological

alterations were consistent with the biochemical and functional data, further supporting the role of prosurvival pathways in mediating HO-1 induced protection.

Results: In-vivo study (Isoproterenol-Induced Myocardial Infarction in rats)

Isoproterenol administration in disease control group led to significant ST-segment elevation, appearance of pathological Q waves, and an increased heart rate (BPM) compared to the normal control group. HO-1 induction by Hemin notably reduced the ST-segment elevation and normalised the Q-wave morphology, along with a significant reduction in heart rate compared to the disease control, suggesting an improvement in myocardial electrical stability. LDH and CK-MB, which are the markers of cardiac injury, were measured in the blood serum. The levels of LDH and CK-MB were markedly elevated in disease control group, confirming the extent of cardiac injury induced by isoproterenol. CK-MB and LDH levels were significantly reduced in the Hemin treated group compared to the disease control group, indicating the protective effect of HO-1 induction against myocardial injury. Malondialdehyde (MDA), which is a marker of lipid peroxidation and oxidative stress increased significantly in the hearts of the animals subjected to isoproterenol. MDA levels were reduced in the hearts of the animals treated with HO-1 inducer Hemin. Superoxide dismutase (SOD) and catalase activity were also reduced in the disease control group indicating reduction of antioxidant defence against oxidative stress. Hemin treatment restored both SOD and catalase activities significantly and thereby antioxidant defence. There was significant increase in the HO-1 activity in the Hemin treated group compared to normal and disease control groups. Increase in HO-1 activity confirmed the induction of HO-1 enzyme by Hemin**.** There was significant reduction in the PI3K activity in the disease control group. Hemin pretreatment significantly increased PI3K activity compared to the disease control group. This finding suggests a potential role of PI3K signaling in HO-1 mediated cardioprotection. Histopathological examination of the heart tissue from the disease control group displayed changes such as myocardial fibre disruption, necrosis, inflammatory cell infiltration, and edema, which are consistent with the myocardial infarction, including. In contrast, cardiac tissue from the Hemin treated group displayed preserved myocardial architecture, reduced necrosis, and reduced inflammatory infiltration. These changes support other findings of HO-1 mediated cardioprotection.

Results: In-vitro study (ISO-induced myocardial injury in H9c2 cardiomyoblasts)

There was significant reduction in the cell viability following ISO exposure, as measured by MTT assay. Cellular damage was also observed following ISO exposure as evident by the elevated level of CK-MB in the cell culture supernatant. There was significant increase in the

cell viability in dose dependent manner with Hemin pretreatment. These changes demonstrating potent cytoprotective effects of HO-1 against ISO-induced injury. There was also significant decrease in the release of CK-MB in cell culture supernatant of Hemin treated groups. Based on these finding it can be confirmed that HO-1 induction protects the cardiomyocytes from injury and also improve survival. There was significant decrease in the Phosphoinositide 3-kinase (PI3K) levels in the H9c2 cell lysate of ISO treated group indicating the inhibitory effect of ISO on PI3K signaling pathway. HO-1 induction by Hemin significantly elevated PI3K levels compared to the ISO-treated group.

Achievements with Respect to the Objectives

Primary Objective:

The primary objective was fulfilled by the successful evaluation of the effects of Heme oxygenase-1 (HO-1) modulation in myocardial infarction and its interaction with key pro-survival signaling pathways through ex vivo, in vivo, and in vitro experimental models of myocardial injury.

Specific Objectives

Ex-vivo experimental approach involved the evaluation of the effects HO-1 modulation in isolated rat hearts subjected to ischemia–reperfusion injury using Langendorff technique. There was significant recovery in cardiac functional, biochemical and histological parameters following HO-1 induction by Hemin. These protective changes were attenuated following HO-1 inhibition. These findings confirmed the protective role of HO-1 in myocardial ischemia–reperfusion injury. In vivo experimental approach involved the evaluation of the effects of HO-1 modulation in isoproterenol-induced myocardial infarction in rats. There was improvement in the ECG patterns, reduction in the cardiac injury markers and oxidative stress, and preservation of cardiomyocyte architecture following HO-1 induction by Hemin. These findings suggest that HO-1 plays an important role to protect myocardium during stressful events. In vitro experimental approach involved the assessment of the effects of HO-1 induction in H9c2 cardiomyoblasts subjected to isoproterenol-induced injury. There was significant improvement in the cell viability and reduced injury of H9c2 cardiomyoblasts following HO-1 induction. These observations support the direct cardioprotective role of HO-1 at the cellular level. HO-1 induction led to consistent cardioprotection in the form of improvement in the cardiac functional, biochemical, and histopathological parameters across all experimental models. These findings reinforce the therapeutic potential of HO-1 modulation

in myocardial injury. HO-1 modulation was achieved through the use of Hemin and zinc protoporphyrin IX (ZnPP IX) to elucidate the effects of HO-1 induction and inhibition, respectively. Significant cardioprotection was achieved through the HO-1 induction across various models. HO-1 inhibition abolished protective effects, thereby establishing a causal relationship between HO-1 activity and cardioprotection. Mechanistic exploration revealed the involvement of multiple pro-survival signaling pathways such as PI3K, PKC, NOS, and KATP in mediating cardioprotective effects following HO-1 induction. Among these pathways, PI3K signaling was found to exert significant cardioprotection following HO-1 induction. The use of pathway-specific inhibitors demonstrated that inhibition of these pathways significantly reduced the protective responses at varying degree, indicating their contribution to HO-1-mediated cardioprotection. Collectively, the findings of this study provide integrated experimental evidence supporting the cardioprotective role of HO-1 and its interaction with key pro-survival signaling pathways in myocardial injury.

Conclusion

Current study systematically evaluated the effects of Heme oxygenase-1 modulation across ex-vivo, in-vivo and in-vitro models of myocardial injury and its potential to activate prosurvival mechanisms. HO-1 induction by hemin improved cardiac functional, biochemical and histological parameters and thereby increased cardiomyocyte survival and reduced damage. HO-1 inhibition led to reversal of change in the aforementioned parameters which confirmed the cardioprotective role of HO-1. Moreover, mechanistic evaluation using pathway specific inhibitors of PI3K, PKC, NOS, and K_{ATP} channels confirmed that HO-1 mediated cardioprotection involved activation of multiple prosurvival signaling pathways. Among these pathways, PI3K signaling was found to have pronounced effect on HO-1 mediated cardioprotection, as its inhibition significantly abolished the protective effects of HO-1.

These findings collectively establish HO-1 as an important upstream regulator of cardioprotective mechanisms. Cardioprotective effects across various models highlight the translational relevance of HO-1 and makes HO-1 modulation as a promising therapeutic strategy for myocardial injury and infarction.

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