HHS Public Access

Author manuscript

*Med Hypotheses*. Author manuscript; available in PMC 2021 February 01.

Published in final edited form as:

*Med Hypotheses*. 2020 February ; 135: 109451. doi:10.1016/j.mehy.2019.109451.

# Optimizing cardiac ischemic preconditioning and postconditioning via epitranscriptional regulation

**Richa Saxena**[1,2], **Neal L. Weintraub**[1], **Yaoliang Tang**[1]

[1]Vascular Biology Center, Medical College of Georgia at Augusta University, Augusta, GA, USA

[2]Ardrey Kell High School, Charlotte, NC, USA

## Abstract

Ischemic cardiac preconditioning protects the heart during myocardial infarction by activating critical cardioprotective genes such as eNOS, SOD, and HO-1. Clinical trials only show marginal effects of conventional preconditioning strategies, however, in part due to transient activation of cardioprotective genes. Recent studies have shown that N6-methyladenosine (m6A) mRNA methylation is the most abundant RNA modification in eukaryotes, and governs mRNA stability and, in turn, the level of protein expression. We hypothesize that regulation of m6A mRNA methylation levels of cardioprotective mRNAs will result in stable expression of the cardioprotective proteins, rendering ischemic cardiac preconditioning more robust and reducing infarct size. To test this hypothesis, we will test the effects of introducing m6A methylases/demethylases into ischemic preconditioned/post conditioned hearts and subjecting them to myocardial infarction. We will assess the half-life of key cardioprotective mRNAs (e.g., eNOS, SOD, and HO-1) and cardiac apoptosis to determine which m6A methylases/demethylases have a synergistic effect on cardiac preconditioning.

### Keywords

Epitranscriptome; cardiac preconditioning; cardiac postconditioning; m6A

## Introduction

A leading cause of death worldwide is ischemic heart disease/myocardial infarction, which leads to the progressive loss of cardiomyocytes(1). Despite the advancement of therapies, including medications, percutaneous intervention, and coronary bypass surgery, permanent damage to the myocardium and ensuing cardiac dysfunction is associated with

**Correspondence to**: Yaoliang Tang, MD, Ph.D., FAHA, Professor of Cardiovascular Medicine, Vascular Biology Center, Medical College of Georgia at Augusta University, 1460 Laney Walker Blvd, Augusta, GA, 30912. Telephone: (706)-721-8467, yaotang@augusta.edu.

Conflict of Interest

None to disclose.

approximately 50% 5-year mortality rate in post-MI patients(2). Therefore, an efficient strategy for cardioprotection against myocardial infarction is greatly needed.

## Ischemic Cardiac Preconditioning

Developing a strategy for cardioprotection is especially important because the heart is unable to regenerate myocardium(3). Ischemic cardiac preconditioning, elicited by brief, repetitive cycles of coronary occlusion and reperfusion, allows the cardiomyocytes to adapt to conditions of inadequate oxygen supply and can thus promote cardioprotection and myocardial salvage by preventing cardiomyocyte apoptosis and cell death(4). Exposing myocardium to 15 minutes of ischemia promotes tolerance to a subsequent episode of ischemia, making the patient more likely to survive a myocardial infarction(5). Conceptually, this approach is attractive to reduce infarct size and potentially improve long-term prognosis following myocardial function(6).

Because the timing of myocardial infarction is relatively unpredictable, preconditioning is not a practical cardioprotection strategy. Cardiac postconditioning, which is performed starting at the time of reperfusion, is a much more practical approach(7, 8) since most damage to the heart occurs within the first minutes of reperfusion(9). Brief coronary occlusions performed just at the beginning of reperfusion can help protect against ischemia/reperfusion injury(7) and reduce infarct size(8). Studies performed in patients undergoing coronary angioplasty during myocardial infarction have demonstrated that postconditioning can effectively protect the heart(8). Results from in vivo experiments in animals have demonstrated that postconditioning not only reduces infarct size, but it also reduces reperfusion arrhythmias(9). Mechanistically, these effects are linked to reduced cardiomyocyte apoptosis and diminished triggers of reperfusion injury (i.e., oxidants, proinflammatory cytokines, neutrophil infiltration)(10).

## Proteins Associated with Ischemia

Many ischemic cardiac preconditioning/postconditioning related protective genes have been identified. Nitric oxide synthase, which produces nitric oxide (NO) from L-arginine, is an essential enzyme as NO regulates redox signaling and cellular functions(11). Studies have demonstrated that NO has a protective effect in modulating the severity of ischemic or reperfusion injuries(12). Ischemic preconditioning has been shown to increase NO production. Inhibitors of nitric oxide synthase, which reduce NO levels, also block cardioprotection(11). Therefore, these enzymes are crucial to heart function and its ability to respond and adapt to external stressors like ischemia. Decreased apoptosis leads to a more significant rescue of cardiomyocytes, which yields more efficient and effective cardioprotection.

Consistently, both preconditioning and postconditioning decrease necrosis from ischemia and salvage muscle tissue to preserve proper heart function. Despite the results from early clinical trials, cardiac preconditioning/postconditioning have yet to become widely applied, in large part due to the invasive nature of the procedure. Thus, investigators have exploited the phenomenon of remote preconditioning/postconditioning, wherein ischemia rendered in a distant organ or tissue promotes cardioprotection(13, 14). The results of this study

reinforce the conclusion that the effect of remote preconditioning cannot be measured through ex-vivo experimentation, and therefore, further advancements are needed before the clinical implementation of such procedures(15).

A possible reason for the limited success of postconditioning clinical trials is the transient nature of protective gene expression, which is insufficient to protect most ischemic cardiomyocytes from lethal injury, therefore leading to little cardioprotection.

## RNA Methylation

There are many sites on the mRNA on which methylation and other modifications, such as N1-methyladenosine (m1A), 5-methylcytosine (m5C), and 2'-O-methylation, can affect gene expression and stability of mRNA. (16, 17). The modification of m1A regulates tRNA and rRNA stability(16). The modification of m5C has been found in a variety of different cellular structures, though the exact purpose of methylation at this site remains unknown. It has been speculated that m5C may be associated with RNA transport or metabolic gene regulation (17). N6-Methyladenosine (m6A) methylation is the most prevalent internal post-transcriptional nucleoside modification on mammalian RNA (18-20). Mapping m6A sites has shown that m6A is most concentrated near stop codons and 3' untranslated regions (19). M6a may be triggered by external stimuli or cellular signaling.

The functions of m6A mRNA methylation and demethylation, along with other post-transcriptional modifications, are to control the stability of mRNA. The 5' guanosine triphosphate cap and the 3' polyadenylation tail protect the mRNA against degradation by enzymes and proteins and aid in the transport of the mRNA from the nucleus to the cytoplasm (21). On the other hand, m6A methylation decreases the stability of mRNA. For instance, Hastings demonstrated that m6A methylation could regulate the pace of circadian gene expression and other processes. The presence of m6A methylation determines the speed of multiple cellular processes, such as the rate of mRNA production, clock gene transcription, nuclear transfer, and protein complex formation(22). Demethylation of m6A allows for protracted gene expression by providing mRNA stability and protection from degradation, preserving the nucleotides of the mRNA strands that are necessary for protein synthesis, and allowing for increased gene expression by catalyzing the processes of mRNA production and translation.

## Role of m6A Enzymes: Writers, Readers, and Erasers

Methylation is a reversible process that is modified by effectors ("writers" and "erasers") and by methyl-specific binding proteins ("readers") that recognize chemical marks(19). Writers and erasers regulate gene expression by respectively installing and removing modifications(19). Writer machinery includes METTL3 as well as the writer complex of METTL14, and adaptor proteins (i.e., WTAP, VIRMA, HAKAI), which install methylations at specific sequences. Erasers (i.e., demethylases) that remove these methylation marks include FTO and ALKBH5. "Reading" is facilitated by YTH domain-containing proteins recruited to m6A modification sites that could favor RNA-binding events, along with RNA binding proteins. In areas where m6A regions are dense, a higher concentration of readers is

present. In Table 1, we list the writers, erasers, and readers that have been reported to affect the stability of mRNA in specific cells.

By providing stability and protection to the RNA, these modifications extend the half-life of RNA by preventing its degradation. Therefore, the RNA can sustain gene expression for a more extended period to promote protein synthesis. Some demethylated mRNA strands have a shortened half-life compared to methylated mRNA strands and are therefore unable to efficiently carry out gene expression. Mauer J et al. (23)reported that FTO (an eraser) causes a significant reduction in mRNA stability via demethylating $m^6A_m$.

## Conclusion

In conclusion, the purpose of the study is to optimize the efficiency and effectiveness of ischemic cardiac preconditioning and postconditioning to better protect the heart during myocardial infarction. M6A mRNA methylation and demethylation have been shown to regulate the half-life of mRNA. Regulating m6A methylation of cardioprotective mRNAs will likely result in stable cardioprotective protein expression, making ischemic cardiac preconditioning/postconditioning more efficient and further reducing the mortality and morbidity of myocardial infarction in patients.

## Hypothesis

We hypothesize that regulating m6A mRNA methylation can increase the effect of cardioprotection by prolonging cardioprotective gene expression. Specifically, demethylation will likely result in more efficient ischemic cardiac preconditioning/ postconditioning in the setting of myocardial infarction.

## Discussion

Our hypothesis introduces novel concepts to the field of cardioprotection. N6-methyladenosine (m6A) has received great attention in recent years because it is the most abundant mRNA modification(24), and has been shown to be reversible and involves many cellular processes such as mRNA and miRNA Processing, mRNA localization, inhibition or translational activation(25). Before we can test the effects of m6A modification on ischemic cardiac preconditioning/postconditioning, we need to test this idea in vivo using both small and large animal MI models. To test this hypothesis, we will increase m6A methylation by introducing methylases (i.e., “writers” such as Mettl3, Mettl14 and WTAP) or decrease m6A methylation by introducing demethylases (i.e., “erasers” such as FTO and ALKBH5) to the hearts, and subject them to ischemic preconditioning or postconditioning followed by myocardial infarction.

In order for cardiomyocytes to overexpress methylase (i.e., Mettl3) or demethylases (i.e. FTO or ALKBH5), we can use recombinant adeno-associated virus (rAAV) vectors with a cardiac-specific promoter (e.g., a miniaturized cardiac-specific regulatory cassette (cTnT(455)) including enhancer and promotor portions of the human cardiac troponin T gene (TNNT2)) to control the specific expression of these genes in cardiomyocytes(26).

rAAV is a safe virus with less immune response and has been used in clinical trials in human patients(27).

To study relative stability of mRNA, we will measure the mRNA decay kinetics, in brief, we will first inhibit mRNA transcription by using actinomycin D or α-amanitin treatment, and then we can compare the time course of key cardioprotective mRNAs in preconditioned hearts at regular time intervals before, during, and after heart preconditioning by quantitative RT-PCR (q-RT-PCR)(28). For some mRNA with very short half-life (<1min), the half-life measurement will be very challenged; however, we will measure key cardioprotective mRNAs, such as eNOS with half-life of ~24hrs (29), SOD with half-life of ~37.5min(30), and HO-1 with half-life of ~2hrs(31). If data analysis show preconditioning with decreased mRNA methylation extends the half-life of mRNA and long-lasting protein expression, we will study whether it has more potent effects on cardioprotection, such as reduction of cardiac apoptosis, and infarct size. Moreover, we will perform echocardiography to see whether combining preconditioning with mRNA methylation regulation can preserve heart function better than traditional precondition strategies. If so, it can be inferred that demethylated mRNA allows for better preconditioning, which in turn leads to enhanced cardiac function.

## Implications

Demonstrating that regulating m6A mRNA methylation increases the efficiency of ischemic cardiac preconditioning/postconditioning will help to advance our understanding of the mechanisms that allow the heart to adapt to adverse conditions such as ischemia. Moreover, this research may lead to methodology to therapeutically regulate the amount or speed of gene and protein expression from mRNA. More efficient ischemic cardiac preconditioning/postconditioning will allow the patients to better adapt to such conditions and will significantly increase the chances of survival in subsequent prolonged episodes of ischemia. This research can also be applied to cases related to ischemia in other organs, such as the brain, kidneys, or limbs. Hence, ischemic preconditioning can be applied to patients at risk for stroke, renal cortical necrosis, etc. Similar to the heart muscle; preconditioning can help these organs develop adaptations to ischemia or other stress conditions. Thus, with further research, our hypothesis may yield better treatment in the future to prevent organ failure.

## Acknowledgment

Y.T. is supported by American Heart Association Grant-in-Aid 16GRNT31430008 and NIH grants AR070029, HL086555, and HL134354; N.L.W. is supported by NIH grants AR070029, 126949, HL142097 and HL134354.

## References

1. Hashimoto H, Olson EN, Bassel-Duby R. Therapeutic approaches for cardiac regeneration and repair. Nat Rev Cardiol. 2018;15(10):585–600. [PubMed: 29872165]
2. Law MR, Watt HC, Wald NJ. The underlying risk of death after myocardial infarction in the absence of treatment. Arch Intern Med. 2002;162(21):2405–10. [PubMed: 12437397]
3. Steinhauser ML, Lee RT. Regeneration of the heart. EMBO Mol Med. 2011;3(12):701–12. [PubMed: 22095736]

4. Hausenloy DJ. Cardioprotection techniques: preconditioning, postconditioning and remote conditioning (basic science). Curr Pharm Des. 2013;19(25):4544–63. [PubMed: 23270554]
5. Gerczuk PZ, Kloner RA. Protecting the heart from ischemia: an update on ischemic and pharmacologic conditioning. Hosp Pract (1995). 2011;39(3):35–43. [PubMed: 21881390]
6. Minamino T Cardioprotection from ischemia/reperfusion injury: basic and translational research. Circ J. 2012;76(5):1074–82. [PubMed: 22504127]
7. Pagliaro P, Penna C. Cardiac postconditioning. Antioxid Redox Signal. 2011;14(5):777–9. [PubMed: 20712411]
8. Staat P, Rioufol G, Piot C, Cottin Y, Cung TT, L'Huillier I, et al. Postconditioning the human heart. Circulation. 2005;112(14):2143–8. [PubMed: 16186417]
9. Valen G, Vaage J. Pre- and postconditioning during cardiac surgery. Basic Res Cardiol. 2005; 100(3): 179–86. [PubMed: 15723155]
10. Vinten-Johansen J, Yellon DM, Opie LH. Postconditioning: a simple, clinically applicable procedure to improve revascularization in acute myocardial infarction. Circulation. 2005;112(14): 2085–8. [PubMed: 16203924]
11. Sun J, Murphy E. Protein S-nitrosylation and cardioprotection. Circ Res. 2010;106(2):285–96. [PubMed: 20133913]
12. Bolli R Cardioprotective function of inducible nitric oxide synthase and role of nitric oxide in myocardial ischemia and preconditioning: an overview of a decade of research. Journal of molecular and cellular cardiology. 2001;33(11):1897–918. [PubMed: 11708836]
13. Shi W, Vinten-Johansen J. Endogenous cardioprotection by ischaemic postconditioning and remote conditioning. Cardiovasc Res. 2012;94(2):206–16. [PubMed: 22323534]
14. Donato M, Evelson P, Gelpi RJ. Protecting the heart from ischemia/reperfusion injury: an update on remote ischemic preconditioning and postconditioning. Curr Opin Cardiol. 2017;32(6):784–90. [PubMed: 28902715]
15. Deja MA, Wiaderkiewicz R, Czekaj P, Czech E, Malinowski M, Machej L, et al. Remote Ischaemic PrEconditioning of Human Myocardium (RIPE): study protocol for a double-blinded randomised controlled trial. Kardiol Pol. 2018;76(1):136–43. [PubMed: 28980297]
16. Chen W, Lin H. Recent Advances in Identification of RNA Modifications. Noncoding RNA. 2016;3(1):1.
17. Jacob R, Zander S, Gutschner T. The Dark Side of the Epitranscriptome: Chemical Modifications in Long Non-Coding RNAs. Int J Mol Sci. 2017;18(11):2387.
18. Dorn LE, Lasman L, Chen J, Xu X, Hund TJ, Medvedovic M, et al. The N(6)-Methyladenosine mRNA Methylase METTL3 Controls Cardiac Homeostasis and Hypertrophy. Circulation. 2019;139(4):533–45. [PubMed: 30586742]
19. Frye M, Jaffrey SR, Pan T, Rechavi G, Suzuki T. RNA modifications: what have we learned and where are we headed? Nat Rev Genet. 2016;17(6):365–72. [PubMed: 27140282]
20. Wang Y, Zhao JC. Update: Mechanisms Underlying N(6)-Methyladenosine Modification of Eukaryotic mRNA. Trends Genet. 2016;32(12):763–73. [PubMed: 27793360]
21. Bechler K Influence of capping and polyadenylation on mRNA expression and on antisense RNA mediated inhibition of gene expression. Biochem Biophys Res Commun. 1997;241(1):193–9. [PubMed: 9405256]
22. Hastings MH. m(6)A mRNA methylation: a new circadian pacesetter. Cell. 2013;155(4):740–1. [PubMed: 24209613]
23. Mauer J, Luo X, Blanjoie A, Jiao X, Grozhik AV, Patil DP, et al. Reversible methylation of m(6)A(m) in the 5' cap controls mRNA stability. Nature. 2017;541(7637):371–5. [PubMed: 28002401]
24. Dai D, Wang H, Zhu L, Jin H, Wang X. N6-methyladenosine links RNA metabolism to cancer progression. Cell death & disease. 2018;9(2):124. [PubMed: 29374143]
25. Tuncel G, Kalkan R. Importance of m N(6)-methyladenosine (m(6)A) RNA modification in cancer. Med Oncol. 2019;36(4):36. [PubMed: 30879160]

26. Kolwicz SC Jr, Odom GL, Nowakowski SG, Moussavi-Harami F, Chen X, Reinecke H, et al. AAV6-mediated Cardiac-specific Overexpression of Ribonucleotide Reductase Enhances Myocardial Contractility. Mol Ther. 2016;24(2):240–50. [PubMed: 26388461]
27. Loring HS, ElMallah MK, Flotte TR. Development of rAAV2-CFTR: History of the First rAAV Vector Product to be Used in Humans. Hum Gene Ther Methods. 2016;27(2):49–58. [PubMed: 26895204]
28. Lugowski A, Nicholson B, Rissland OS. Determining mRNA half-lives on a transcriptomewide scale. Methods. 2018;137:90–8. [PubMed: 29247756]
29. Ho JJD, Robb GB, Tai SC, Turgeon PJ, Mawji IA, Man HSJ, et al. Active stabilization of human endothelial nitric oxide synthase mRNA by hnRNP E1 protects against antisense RNA and microRNAs. Molecular and cellular biology. 2013;33(10):2029–46. [PubMed: 23478261]
30. Bini E, Dikshit V, Dirksen K, Drozda M, Blum P. Stability of mRNA in the hyperthermophilic archaeon Sulfolobus solfataricus. Rna. 2002;8(9):1129–36. [PubMed: 12358432]
31. Bouton C, Demple B. Nitric oxide-inducible expression of heme oxygenase-1 in human cells. Translation-independent stabilization of the mRNA and evidence for direct action of nitric oxide. J Biol Chem. 2000;275(42):32688–93. [PubMed: 11032845]
32. Han J, Wang J-z, Yang X, Yu H, Zhou R, Lu H-C, et al. METTL3 promote tumor proliferation of bladder cancer by accelerating pri-miR221/222 maturation in m6A-dependent manner. Molecular Cancer. 2019; 18(1):110. [PubMed: 31228940]
33. Xu Y, Yuan XD, Wu JJ, Chen RY, Xia L, Zhang M, et al. The N6-methyladenosine mRNA methylase METTL14 promotes renal ischemic reperfusion injury via suppressing YAP1. J Cell Biochem. 2019.
34. Kobayashi M, Ohsugi M, Sasako T, Awazawa M, Umehara T, Iwane A, et al. The RNA Methyltransferase Complex of WTAP, METTL3, and METTL14 Regulates Mitotic Clonal Expansion in Adipogenesis. Mol Cell Biol. 2018;38(16).
35. Yue Y, Liu J, Cui X, Cao J, Luo G, Zhang Z, et al. VIRMA mediates preferential m(6)A mRNA methylation in 3'UTR and near stop codon and associates with alternative polyadenylation. Cell Discov. 2018;4:10. [PubMed: 29507755]
36. Jiang Q, Sun B, Liu Q, Cai M, Wu R, Wang F, et al. MTCH2 promotes adipogenesis in intramuscular preadipocytes via an m(6)A-YTHDF1-dependent mechanism. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2019;33(2): 2971–81. [PubMed: 30339471]
37. Li XC, Jin F, Wang BY, Yin XJ, Hong W, Tian FJ. The m6A demethylase ALKBH5 controls trophoblast invasion at the maternal-fetal interface by regulating the stability of CYR61 mRNA. Theranostics. 2019;9(13):3853–65. [PubMed: 31281518]

**Table 1.**

| Effector | Type of Effector | Expression | Cell(s) | Targeted genes | mRNA stability and protein expression | Ref. |
|---|---|---|---|---|---|---|
| **METTL3** | Writer | Increased | bladder cancer EJ & T24 cells | Pri-miR221/222 | ↑DGCR8 mediated recognition. | (32) |
| **METTL14** | Writer | Decreased | Acute kidney injury | YAP1 | ↑ stability | (33) |
| **WTAP** | Writer | Decreased | 3T3-L1 | Ccna2 | ↓ expression | (34) |
| **VIRMA** | Writer | Decreased | HeLa cells | IGFBP5 Notch1 | ↑ stability | (35) |
| **FTO** | Eraser | Increased | Transcription start sites | MTCH2 | ↓ expression | (36) |
| | | Decreased | Transcription start sites | miR-155 targeted genes | ↑ stability | (23) |
| **ALKBH5** | Eraser | Increased | HTR-8& JEG-3 | CYR6I | ↓ expression | (37) |
| | | Decreased | | | ↑ stability | |
| **YTHDF1** | Reader | Decreased | Porcine intramuscular preadipocytes | MTCH2 | ↓ expression | (36) |