Developing Tools to Study the Role of Mitochondria Dynamic in Myocardial Infarction

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Introduction

Cardiovascular diseases (CVDs)

- ✓ CVDs are a leading cause of mortality and morbidity.
- ✓ Myocardial infarction (MI, a.k.a *heart attack*), is caused also by acute ischemia (Fig. 1).
  - ✓ MI is treated by percutaneous coronary intervention with the aim of coronary reperfusion.
  - ✓ Yet, MI symptoms may worsen by reperfusion as the latter may attenuate mitochondrial dysfunction.

Mitochondrial homeostasis

- ✓ Mitochondrial function is essential for maintaining cellular metabolic homeostasis.
- ✓ Mitochondria homeostasis (Fig. 2) is controlled by specialized proteins, such as Pink1 (Phosphatase and tensin homolog (PTEN)-Induced Putative Kinase Protein 1), and Mfn2 (Mitofusin 2).

Hypothesis and aims

We hypothesize that Pink1/Mfn2 protein-protein interaction (PPI) may be critical for mitochondrial homeostasis.

Therefore, we developed a peptide that specifically intervenes with Pink1/Mfn2 PPI and studied its effects in physiological and pathological conditions *in vitro* and *in vivo*.

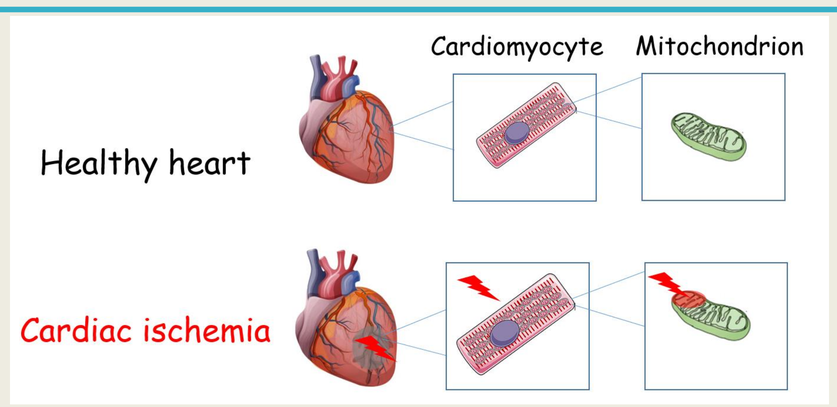


Figure 1: Into a heart in physiological and **pathological** conditions.

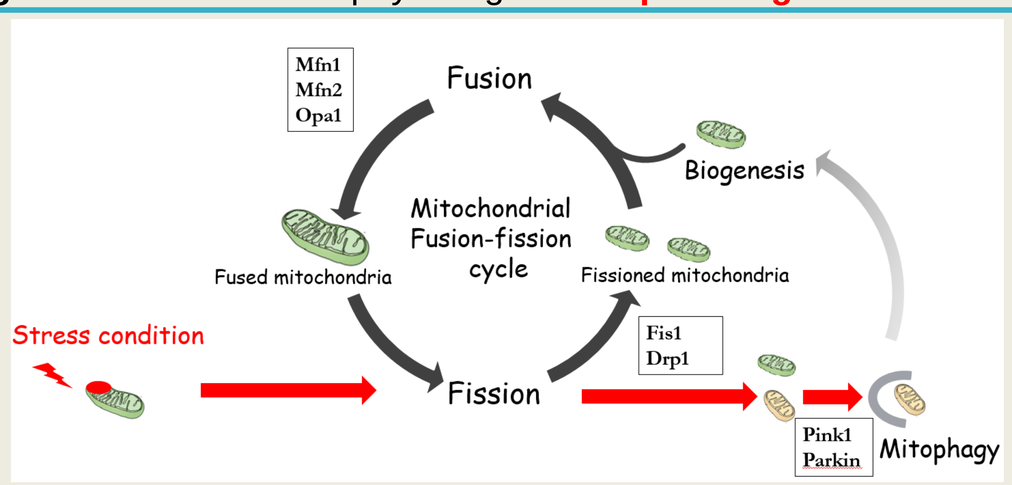


Figure 2: Mitochondrial homeostasis in physiological and **pathological** conditions.

Methods

- We developed a peptide that regulates Pink1/Mfn2 PPI (Fig. 3) and studied its effect on physiological and pathological conditions.
- Cells studies: H9c2 cells were treated with $CoCl_2$, an inducer of ischemia, and cells viability was measured.
- Animal studies: Rats underwent ischemia/reperfusion (I/R) injury, by 30 min surgical ligation of their left anterior descending artery. The hearts were stained with Tetrazolium chloride (TTC) and analyzed for the size of the damaged tissue. Creatinine kinase (CK) indices were taken from the blood.

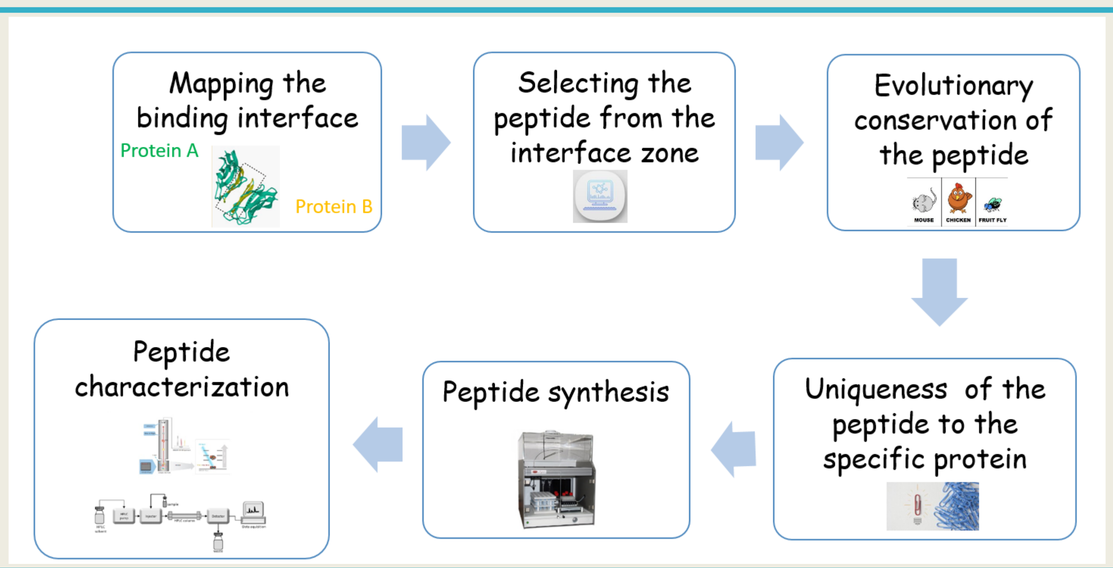


Figure 3: Rational design of peptides that regulates Pink1/Mfn2 PPI

Results

- ❖ We have shown that there is PPI between Pink1/Mfn2.

PPI	Kd (nM)
Pink1 / Mfn2	1
Mfn2 / BSA (CTRL)	13,725

- ❖ Peptide CVP-014 was designed to inhibit Pink1/Mfn2 PPI.
- ❖ Cells studies: CVP-014 increased H9c2 viability under ischemia (Fig. 4).
- ❖ Animal studies: CVP-014 decreased infarct size and CK by > 50% (Fig. 5).
- ❖ Animal studies: CVP-014 was found to be safe in toxicity studies.

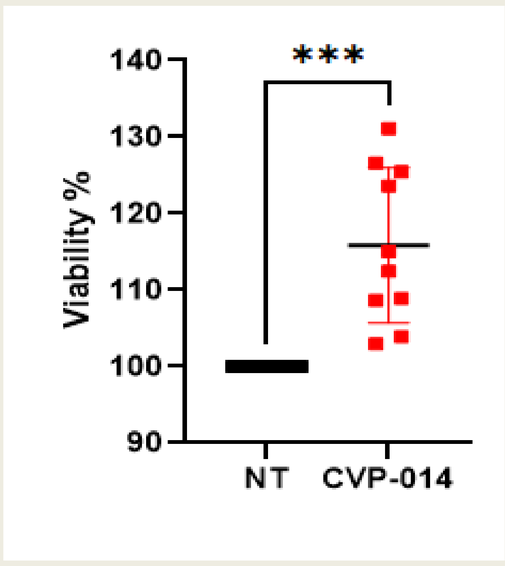
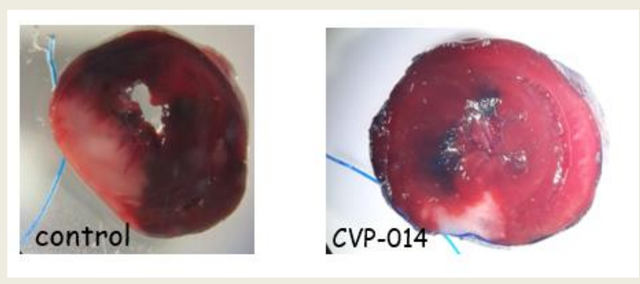
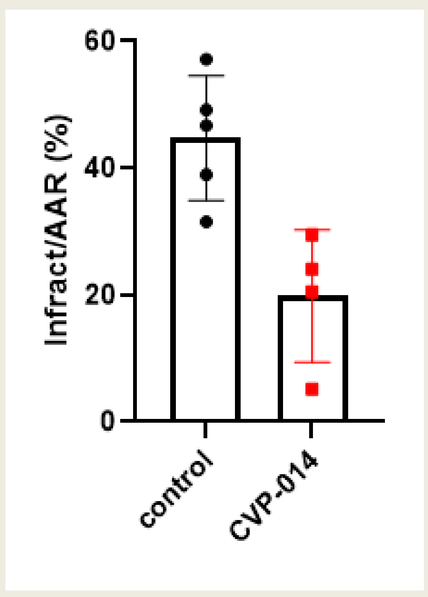


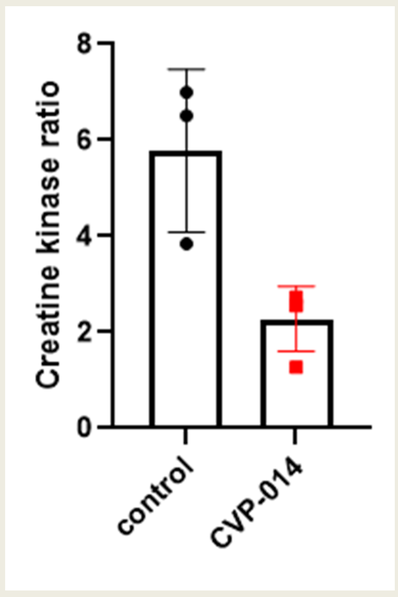
Figure 4: Percentage of vitality of H9c2 cells under ischemia in the presence of the peptide.

Figure 5: Animal studies :
A. Representative cardiac sections, TTC assay.
B. Percentage of infracted area from area at risk (AAR), TTC assay.
C. Creatine kinase blood level ratio final/base line measurements.



Control n=5
CVP-014 n=4





Conclusions

- Our study provides a selective tool to study the mitochondria process and homeostasis, as well as a pharmacological tool for therapeutic applications for the treatment of MI.
- CVP-014 has a potent efficacy on the cardiac tissue, as it reduces the infarct size and cardiac damage (CK).
- Further, research is essential to fully decipher the mechanism of action at the physiological level.

Clinical implications

- As the mitochondria are considered the weakest link in the cardiomyocytes, during hypoxia, its preservation bares highly importance.
- Early, post-MI, mitochondrial treatments will provide a powerful clinical tool that will reduce cellular and tissue damages, organ (cardiac) dysfunction, and further cardiovascular morbidity, which is associated with hospitalizations and eventually mortality.
- Currently, there are no such treatments in the clinical arena, thus this new approach probably holds a synergistic effect on the current clinical protocols.