Undergraduate Students - Populations, Health and Wellness

In Vivo Investigation of Cardiac Benefits of Sodium Glucose Cotransporter Inhibition Using the Zebrafish Model

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Abstract

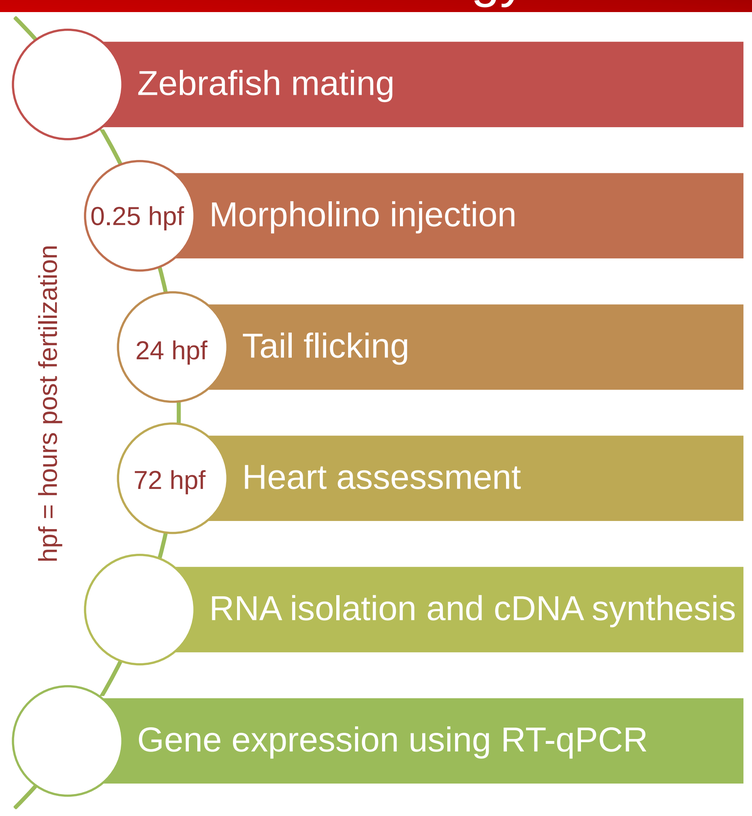
Type 2 diabetes mellitus (T2DM) affects >16% of adults in Qatar. Newly emerging class of anti-diabetic drugs focuses on SGLT inhibition were observed to reduce CVDs risks in diabetic patients. Up to date, the mechanism contributing to the CV benefits remains unrevealed. Zebrafish embryos were injected with different morpholinos to knockdown SGLT genes and study their effects on cardiac parameters. SGLT1 inhibition caused the most severe effects on zebrafish embryos with survival rate ~10 %. It also caused tube-like structured heats with edema, affecting significantly the cardiac output and diameter, and increased cardiac markers expressions. Analysis acquired correlates with literature data of SGLT1 predominant expression in heart tissues.

Introduction

Until today, the exact mechanism of SGLT inhibition effects on CV tissues remains unclear. Therefore, this research aims to understand:

- Role of SGLT genes expression in normal individuals.
- Analyze the impact of SGLT1 and SGLT2 absence on the development of heart disease and function.
- Identify the relationship between SGLT1 and SGLT2 inhibition and compensatory effects.

Methodology



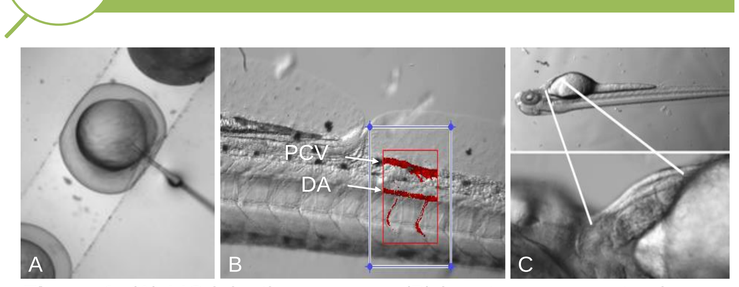


Figure 1. (A) MO injection process, (B) heart assessment using PCV and DA, (C) zebrafish heart location.

Results

I. SGLT inhibition has significantly reduced the survival rates of zebrafish embryos, especially with SGLT1 inhibition

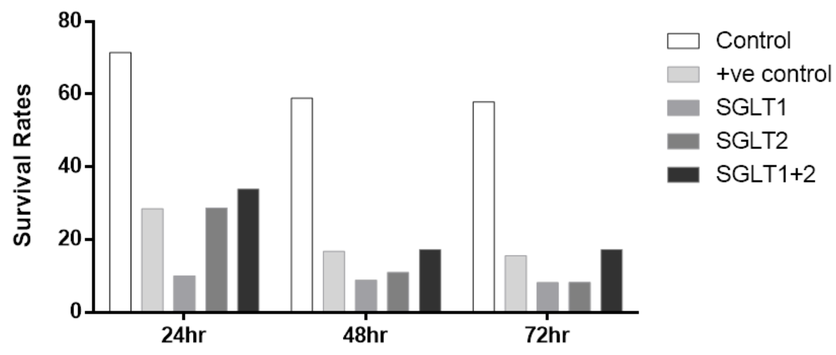


Figure 2. SGLT inhibition with MO has significantly affected the survival rates of the embryos from the first 24 hpf, especially SGLT1 inhibition.

II. Tail flicking has been significantly reduced with SGLT1 MO compared to the negative control

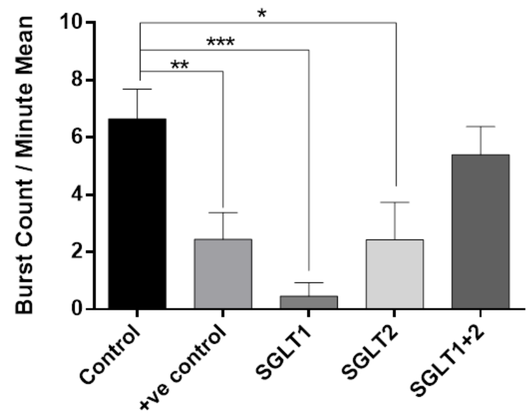


Figure 3. Tail flicking of embryos following gene knockdown done using DanioScope software. Analysis was by one-way-ANOVA with Sidak post hoc test. *p<0.05, **p<0.01, ***p<0.001 (n=20±6).

III. SGLT inhibition has affected heart structure and embryo development

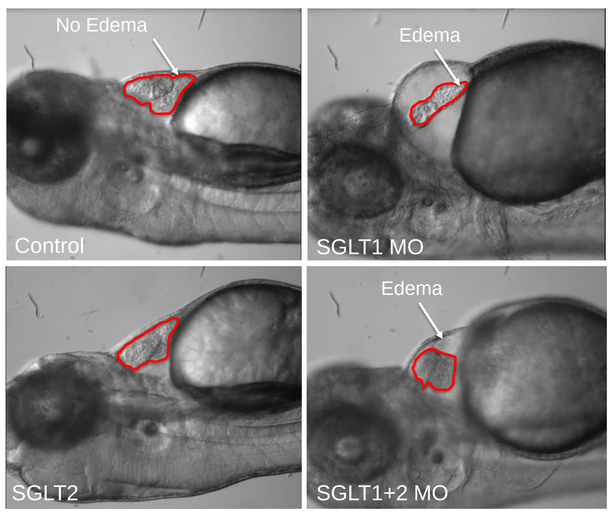


Figure 4. SGLT inhibition has significantly affected the heart structure in SGLT1 inhibition where it became tube-like with edema. SGLT2 inhibition resulted in a slightly elongated heart. While SGLT1+2 resulted in slight edema with no visible chambers.

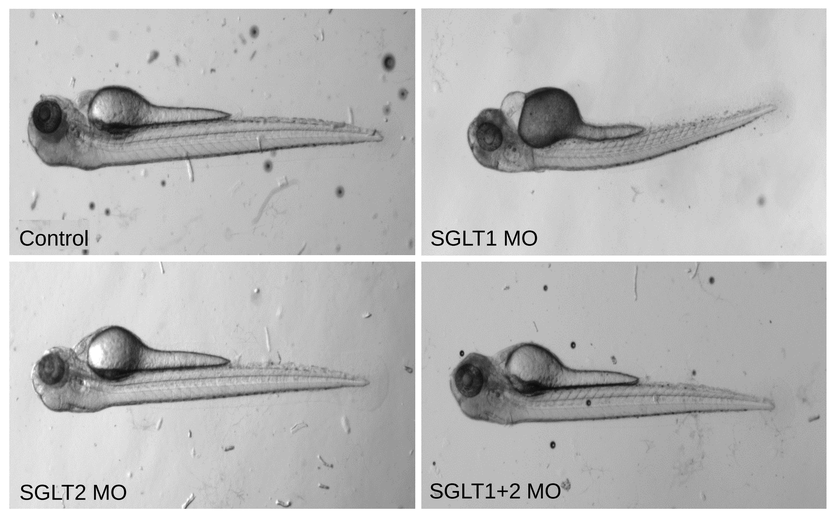


Figure 5. The effect of SGLT inhibition on full embryo size and morphology. The sizes of injected zebrafish embryos showed under-development after SGLT inhibition.

IV. SGLT1 inhibition has significantly affected cardiac parameters

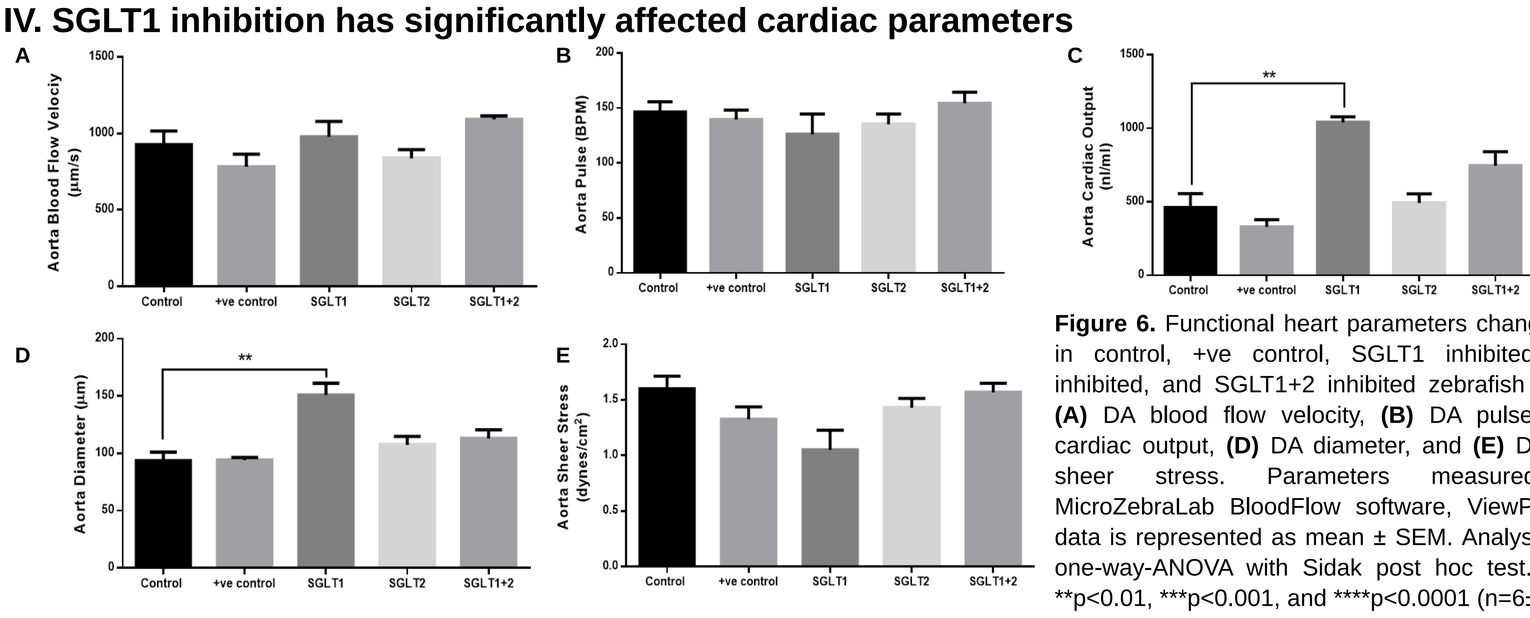


Figure 6. Functional heart parameters changes of DA in control, +ve control, SGLT1 inhibited, SGLT2 inhibited, and SGLT1+2 inhibited zebrafish embryos. **(A)** DA blood flow velocity, **(B)** DA pulse, **(C)** DA cardiac output, **(D)** DA diameter, and **(E)** DA cardiac sheer stress. Parameters measured using MicroZebraLab BloodFlow software, ViewPoint. All data is represented as mean ± SEM. Analysis was by one-way-ANOVA with Sidak post hoc test. *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001 (n=6±1).

V. SGLT1 inhibition has significantly increased cardiac markers expressions

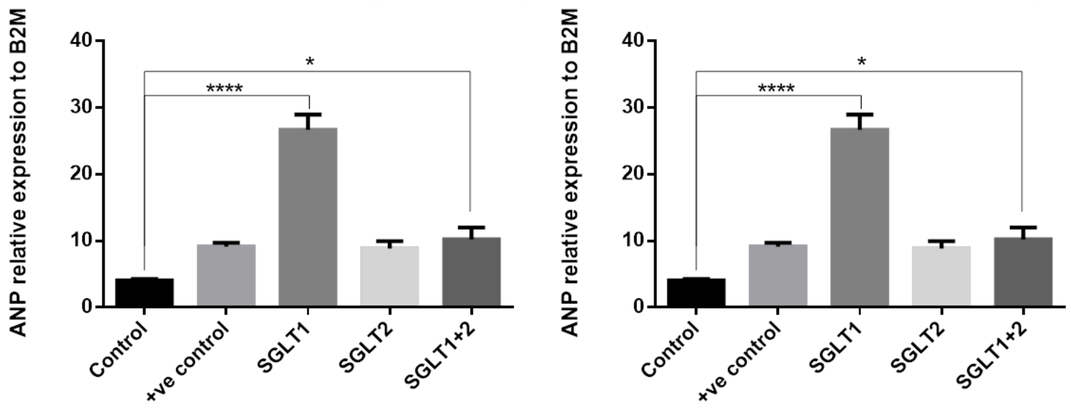


Figure 7. BNP and ANP relative gene expression to reference gene B2M in experimental groups. One-way ANOVA with Sidak post hoc test for multiple comparison. *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001 (n=3).

Conclusion

- In comparison to wild type, SGLT1 knockdown triggered a delay in heart development, reduce vessel diameter and overall cardiac output. SGLT1 MO showed significant effects on structural and functional heart parameters with increased cardiac markers expressions that correlates with literature data of SGLT1 predominant expression in the heart.
- SGLT2 MO resulted in less severity on cardiac function since the expression is lower in heart tissues.
- SGLT1+2 MO has the least effect on cardiac parameters. We will increase the MO concentration to see if higher concentrations will result in phenotype without remarkable under-development of the embryos.

Resources

1. Chen J, Williams S, Ho S, Loraine H, Hagan D, Whaley JM, et al. Quantitative PCR tissue expression profiling of the human SGLT2 gene and related family members. Diabetes Ther. 2010;1(2):57-92.
2. Zakaria ZZ, Benslimane FM, Nasrallah GK, Shurbaji S, Younes NN, Mraiche F, et al. Using Zebrafish for Investigating the Molecular Mechanisms of Drug-Induced Cardiotoxicity. Biomed Res Int. 2018;2018:1642684.
3. Yalcin HC, Amindari A, Butcher JT, Althani A, Yacoub M. Heart function and hemodynamic analysis for zebrafish embryos. Dev Dyn. 2017;246(11):868-80.