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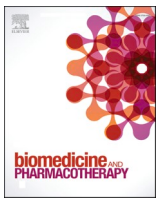

Empagliflozin attenuates hypoxia-induced heart failure of zebrafish embryos via influencing MMP13 expression

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ABSTRACT

Background: Today, sodium glucose co-transporter 2 (SGLT2) inhibitors are more than diabetes drugs. They are also indicated in chronic heart failure (HF) treatment in both diabetic and non-diabetic patients, independently of the ejection fraction. Multiple mechanisms have been suggested behind the cardioprotective effects of SGLT2 inhibitors. However, the underlying mechanisms still remain largely unexplored. Here, we used a zebrafish embryo model to search for new potential players whereby SGLT2 inhibitors attenuate HF.

Methods: HF in zebrafish embryos was caused exposing them to chemically induced hypoxia. As a SGLT2 inhibitor, we used empagliflozin. Its effect on hypoxia-induced HF of the embryos was evaluated using video microscopy and calculation of fractional shortening (FS) of embryos´ hearts. RT-qPCR of brain natriuretic peptide (*bnp*) expression was also used to examine empagliflozin´s effect on HF. Transcriptome analysis of total RNA of the embryos was performed to search for new potential mechanisms contributing to the beneficial effect of empagliflozin on HF.

Results: Empagliflozin significantly attenuated hypoxia-induced HF of zebrafish embryos as shown with improved FS of the hearts and decreased *bnp* expression. Transcriptome analysis revealed that the improvement of HF in response to empagliflozin was accompanied with decreased *matrix metalloproteinase 13a* (*mmp13a*) expression. Treatment of hypoxia-induced embryos with MMP13 inhibitor ameliorated the impaired heart function accordingly to the effect of empagliflozin. MMP13 inhibitor was not toxic to the embryos.

Conclusions: Our study shows that empagliflozin´s favorable effect on attenuating HF is mediated via MMP13. MMP13 provides a novel option when developing new therapeutics for HF treatment.

1. Introduction

Sodium glucose co-transporter 2 (SGLT2) inhibitors, originally developed as glucose-lowering drugs for the treatment of type 2 diabetes mellitus, are currently known to be much more than that. They are also drugs for chronic heart failure (HF) treatment, independently whether the patient has diabetes or not [1,2]. The breakthrough of SGLT2 inhibitors in diabetes treatment took place in 2015, when the EMPA-REG-OUTCOME trial was published [3]. In that trial, the SGLT2 inhibitor empagliflozin was shown to significantly lower the risk of death from cardiovascular causes, death from any cause and additionally, hospitalization for HF among patients with type 2 diabetes at high risk for cardiovascular events. Subsequently, also canagliflozin, dapagliflozin and ertugliflozin were shown to reduce the risk for HF events [4–8]. Today we know that SGLT2 inhibitors are able to decrease the risk for hospitalization due to HF even in non-diabetic patients exhibiting HF with reduced or even preserved ejection fraction [9,10]. This is also true for sotagliflozin that in addition to inhibiting SGLT2 possesses a marked SGLT1 inhibitory activity [11]. Today it is well established that SGLT2 inhibitors are also effective at slowing chronic kidney disease in patients with or without diabetes [12,13].

The mechanisms by which SGLT2 inhibitors lower the risk for HF are presently not exactly known. However, because these drugs exert their beneficial effects on heart function in patients both with and without diabetes, it is obvious that mechanisms other than the improvement of glycemic control play major roles. The proposed mechanisms of SGLT2

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inhibitors in reducing HF include e.g. the following ones: impact on the metabolism of fatty acids and ketone bodies, modulation of mitochondrial function, negative sodium balance and redistribution of sodium ions, reduction in plasma uric acid concentration, stimulation of erythropoiesis, effects on hypoxia inducible factors, sirtuin 1 and autophagy, impact on glucagon secretion, weight reduction, and lowering of blood pressure [14–16]. Furthermore, in recent years the anti-inflammatory, anti-fibrotic and anti-apoptotic effects of SGLT2 inhibitors on the heart, have received attention [17–19]. Additionally, effects of SGLT2 inhibitors on iron metabolism have been suggested to play a role in improving clinical outcomes in patients with HF via activating hematopoiesis through the reduction of hepcidin, a molecule that inhibits iron absorption in the gut and iron mobilization from macrophages [20]. Nonetheless, it is important to keep in mind that SGLT2 inhibitors also improve the quality of life in patients independently of their glycemic status [21].

Here, we used a zebrafish model of HF by simulating HF through exposing zebrafish embryos to hypoxic conditions. This way we aimed to find out new potential mechanisms contributing to thus far partially unknown beneficial effects of SGLT2 inhibitors on HF.

2. Material and methods

2.1. Zebrafish care and breeding

Adult zebrafish were placed into mating tanks and after natural spawning, the fertilized eggs were collected and cultured in E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, 0.33 mM $MgSO_4$) supplemented with 0.2 mM phenylthiourea (Sigma-Aldrich) at 28.5 °C. Experiments were performed under the license of MMM/465/712–93 issued by the Finnish Ministry of Agriculture and Forestry and carried out in the Zebrafish core facilities, Turku Bioscience Centre, Turku Finland.

2.2. Induction of HF and subsequent treatments of zebrafish embryos

Zebrafish embryos (n=20) were cultured on 6-well plates in E3 medium at 28.5℃ for 48 h (Fig. 1. A), after which they were treated with empagliflozin (50 or 100 μM) or MMP13 inhibitor (40 μM). Dimethyl sulfoxide (DMSO) (1 %) was used as a vehicle control in the E3 medium. Before the experiments in which MMP13 inhibitor was used, the toxicity of the inhibitor to the embryos was tested in a maximum tolerated dose test (Fig. S1). Empagliflozin was purchased from Selleck Chemicals (Houston, TX, USA). MMP13 inhibitor (CAS 544678–85–5) was obtained from Merck (Darmstadt, Germany).

At the time point 72 hours post fertilization (hpf), the embryos (excluding the embryos in normoxia) were exposed to hypoxic conditions for 25 min as follows: The hypoxic medium was produced by boiling E3 medium for five minutes, followed by adding Na_2SO_3 (1 mg/ml) as oxygen scavenger [22], dispensing hot medium into 30 ml glass vial, cooling the solution in a 28.5℃ water bath, and neutralizing the pH to 7. Once the hypoxic medium was equilibrated to 28.5℃, the embryos were gently transferred with a glass pipette into glass vials containing hypoxic medium. After 25 min incubation in hypoxic medium, the embryos were transferred to petri dishes containing normoxic E3 medium, followed by washing with normoxic E3 medium and finally transferred to normoxic E3 medium containing empagliflozin (50 or 100 μM) or MMP13 inhibitor (40 μM) or vehicle control DMSO (1 %), and incubated

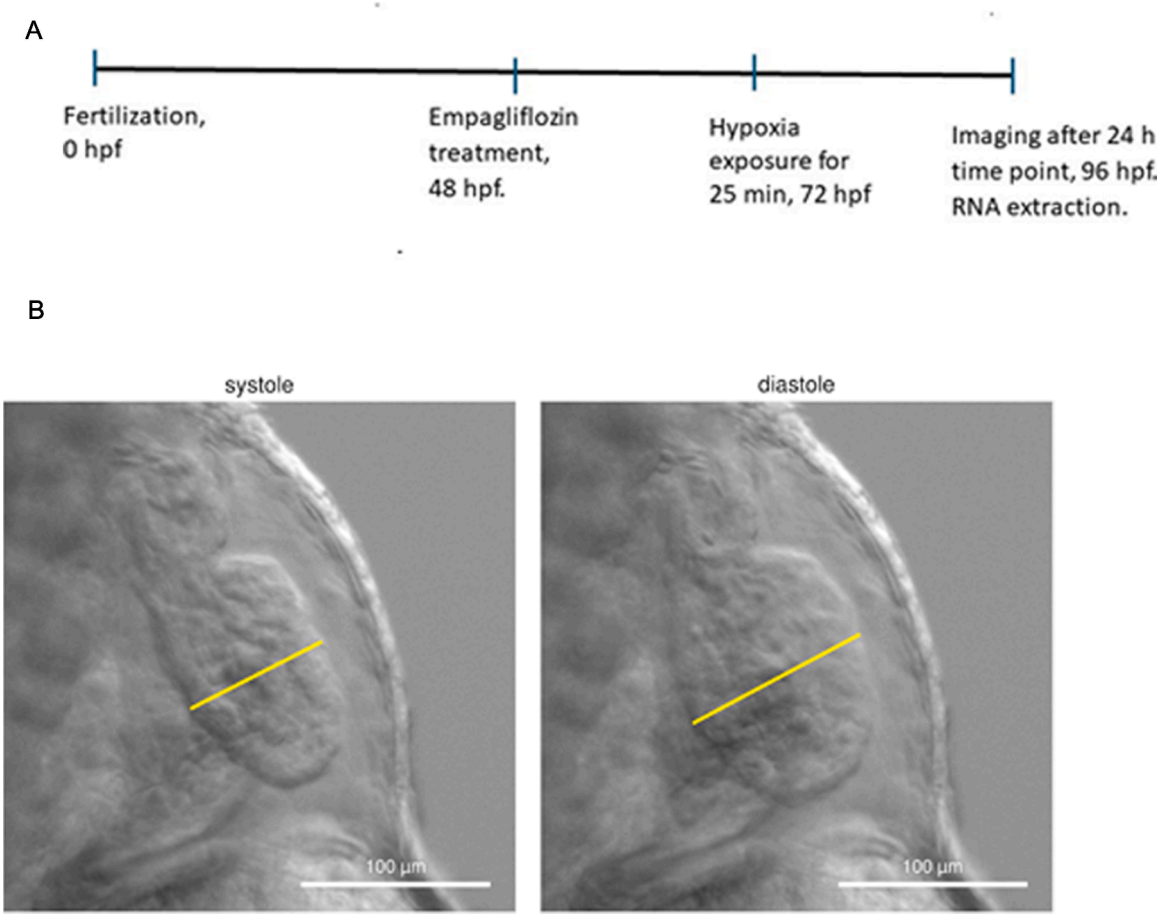

Fig. 1. A. Timeline of the experimentation with zebrafish embryos (hpf, hours post fertilization).**B.** Representative video captures of the beating heart of a zebrafish embryo. The yellow lines show the small diameter of the ventricle in systole (left) and in diastole (right).

at 28.5 ℃ until analyzed. The rationale behind the concentration of empagliflozin applied in the present study has been described in our previous work with zebrafish embryos [23].

2.3. Analysis of the heart function of zebrafish embryos

The embryos were imaged with video microscopy using Zeiss axioZOOM stereomicroscope (Zeiss, Jena, Germany) in connection with CCD camera (Hamamatsu, Japan). The magnification was 80x, exposure time was 25 ms, frame size 512 ×512, pixels 6.5 ×6.5 μm and binning 1 ×1. From the videos, representative images were captured, followed by brightness and contrast adjustments. The images were analyzed using the measure tool in ImageJ 2.3.0 open-source platform for biological-image analyses to acquire the values of the ventricular chamber small diameters in both systole and diastole (Fig. 1. B).

Embryos that died during the hypoxia exposure were counted and excluded. The remaining embryos were anesthetized using tricaine 0.5 % (ethyl 3-aminobenzoate methane sulfonate salt, 200 mg/l, Sigma-Aldrich, Saint Louis, Missouri, USA) in E3 medium. The embryos were allowed to acclimatize to room temperature and short videos of the beating hearts were acquired. The videos (n=28 of each group, except for normoxic group, n=25) were analyzed and the FS was calculated. The data presented are from pooled experiments. The data were tested for normality and variance. The ventricular FS was calculated (Eq. 1) from the video microscopy recorded small diameters of the ventricular chamber according to the method of Hoage et al., 2011 [24].

Equation (1): FS = (*small diameter at diastole – small diameter at systole*) / *small diameter at diastole*

Furthermore, the heart function of the zebrafish embryos was also evaluated determining brain natriuretic peptide (*bnp)* expression in different conditions using RT-qPCR. Briefly, the embryos were collected for RT-qPCR gene expression analysis 24 h after hypoxic exposure and homogenized using a motorized pellet pestle (Sigma-Aldrich). Total RNA was extracted using the Nucleospin kit for RNA purification (Macherey-Nagel GmbH, Germany). RT-qPCR was carried out using SensiFAST™ cDNA Synthesis Kit (Bioline) and SYBR® Select Master Mix for CFX (Applied Biosciences) as triplicate reactions in CFX96 Touch Real-Time PCR Detection System (BioRad, USA). All procedures were performed according to the instructions provided by the manufacturers. Gene-specific primers were designed using NBCI Primer BLAST or obtained from literature. For RT-qPCR, the primers were optimized for efficiency and checked for the appropriate product size using agarose gel electrophoresis The expression of *bnp* was normalized to ribosomal protein L13a (Rpl13a) mRNA expression and quantified as relative to control using the relative ΔΔCq method. The primers for *bnp* and *rp113a* were as follows: *bnp*: Fw: TGTTTCGGGAGCAAACTGGA, Rv: GTTCTTCTTGGGACCTGAGC; *rpl13a*: Fw: GGCGGACCGATTCAATAAGGTTCTGATCATTG, Rv: CCAGAGATGTTGATACCCTCACACCTCAC.

2.4. Transcriptome analysis

To find out potential mechanisms behind the beneficial effect of empagliflozin on the hypoxia- induced HF of zebrafish embryos, transcriptome analyses of the total RNA of the embryos were performed. Briefly, the embryos were collected at 96 hpf (n = 20 per sample), lysed and their total RNA was extracted with Nucleospin RNA purification kit (Macherey-Nagel GmbH, Germany) according to the manufacturer's instructions. The stranded RNA sequencing analyses were performed for samples from normoxic, hypoxia-treated and empagliflozin-treated embryos (n = 4 in each group), and the subsequent transcriptome analyses were performed at the Finnish Functional Genomics Centre, Turku Bioscience Centre, Turku, Finland (https://bioscience.fi/services/functional-genomics/services/). The data analysis was performed at the Medical Bioinformatics Centre, Turku Bioscience Centre https://bioscience.fi/services/bioinformatics/). The threshold value used in the statistical analysis was fc > 2 and the false discovery rates (fdr) of 0.05.

2.5. Apoptosis analysis using acridine orange cell death assay

Zebrafish embryos subjected to hypoxia-reperfusion or normoxia and treated with empagliflozin or MMP13 inhibitor for 6 h were used in acridine orange (AO) assay. The embryos were anesthetized with 200 mg/l tricaine and stained with 2 μg/ml AO for 30 min. Next, excess AO was washed out with 3 washes with E3. Prior to imaging 1000 mg/l of tricaine was added to stop the heart beat for imaging. Embryos were imaged with Zeiss AxioZoom fluorescence stereomicroscope using reflected white light and fluorescence imaging with excitation: bandpass (BP) 470/40 nm and emission: BP 525/50 nm. Images were captured with 1.0x PlanApo Z, NA0.125 objective and Hamamatsu sCMOS Orca Flash4.0 LT + camera. Fluorescence intensity on heart area was measured using ImageJ/FIJI.

2.6. Reactive oxygen species activity using glutathione assay with monochlorobimane

Zebrafish embryos subjected to hypoxia-reperfusion or normoxia and treated with empagliflozin or MMP13 inhibitor for 24 h were used in monochlorobimane (mBCI) assay. The embryos were anesthetized with 200 mg/l tricaine and stained with 100 μM mBCI (Sigma-Aldrich) for 30 min. Next, excess dye was washed out with 3 washes with E3. Before imaging heart beat was stopped with addition of 1000 mg/l tricaine. Embryos were imaged with Zeiss AxioZoom fluorescence stereomicroscope using reflected white light and fluorescence imaging with excitation: G365nm and emission: BP 445/50 nm. Images were captured with 1.0x PlanApo Z, NA0.125 objective and Hamamatsu sCMOS Orca Flash4.0 LT + camera. Integrated fluorescence intensity on heart area was measured using ImageJ/FIJI.

2.7. Statistical analyses

Statistical analyses were carried out using GraphPad Prism 10 software. First, outliers were detected and removed using ROUT's algorithm. The data was analyzed for normality using D'Agostino and Pearson's test. Next, one-way ANOVA with Dunnett's post-hoc test was performed (Figs. 1–4).

Data from fluorescence staining of zebrafish embryos were analyzed using one-way ANOVA following post-hoc testing with fdr of 0.05 using two-stage linear step-up procedure using GraphPad Prism 10 (Figs. 5–6).

3. Results

3.1. Effect of empagliflozin on the heart function of zebrafish embryos under hypoxic conditions

To analyze the effect of empagliflozin in vivo, we utilized a hypoxia-induced HF model in zebrafish embryos [22]. The heart function of zebrafish embryos was estimated by measuring the small diameter of the ventricular chamber of the heart of zebrafish embryos both in systole and in diastole, i.e., by determining fractional shortening (FS) of the hearts (Fig. 1. B). In hypoxic conditions FS of the hearts decreased statistically significantly compared to that in normoxia indicating that hypoxia induced a marked HF in the embryos (Fig. 2. A). Treatment of the embryos subjected to hypoxia with empagliflozin returned the decreased FS almost to the same level as it was in normoxia (Fig. 2. A). Thus, empagliflozin was able to attenuate the harmful effect of hypoxia on embryos' heart function. The FS results were confirmed by determining *bnp* expression in zebrafish embryos using RT-qPCR. Exposure of the embryos to hypoxia increased the expression of *bnp* approximately 5-fold compared to that in normoxia. Concomitantly to the FS results, empagliflozin almost completely abolished the increase in *bnp*

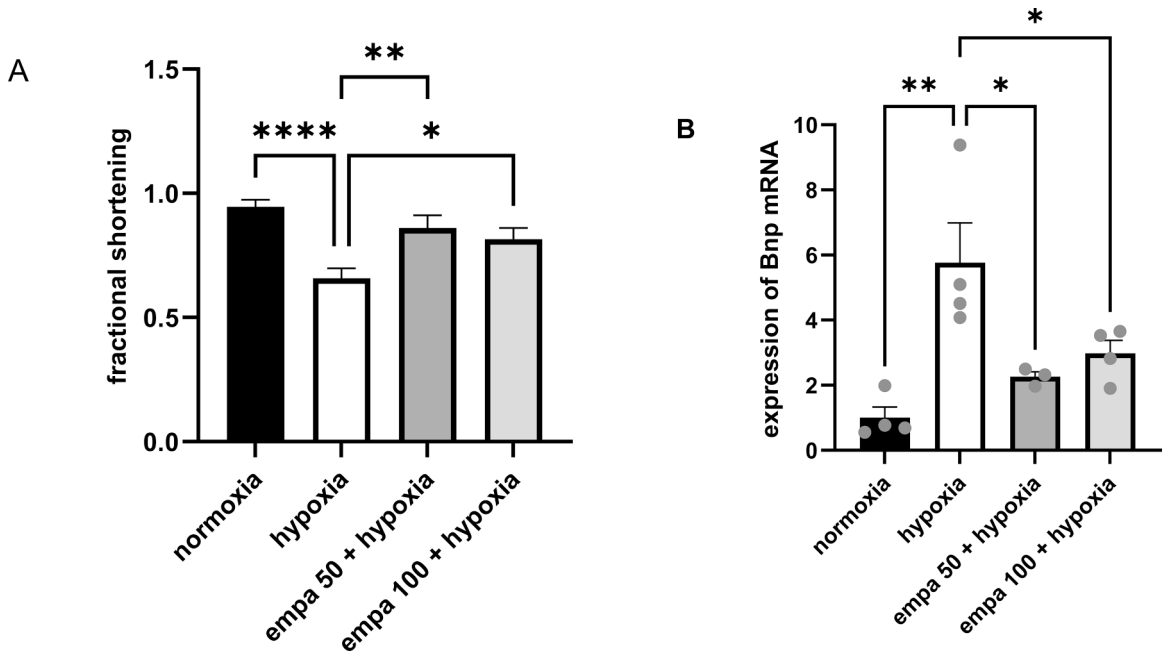

Fig. 2. A. Impact of empagliflozin on zebrafish embryos' hearts under hypoxic exposure. Fractional shortening (FS) of the beating hearts of zebrafish embryos was measured in normoxia and hypoxia, as well as in hypoxia after treatment with empagliflozin (50 and 100 μM). **** P<0.0001, *** P<0.001, **P<0.01, *P<0.05. Capped bars on top of the columns indicate standard error of the means.**B.** Relative expression of Bnp determined with RT-qPCR of hypoxia-exposed zebrafish embryos treated with empagliflozin (50 μM or 100 μM). Bnp expression was downregulated in response to empagliflozin treatment. *** P<0.001, **P<0.01, *P<0.05. Capped bars on top of the columns indicate standard error of the means.

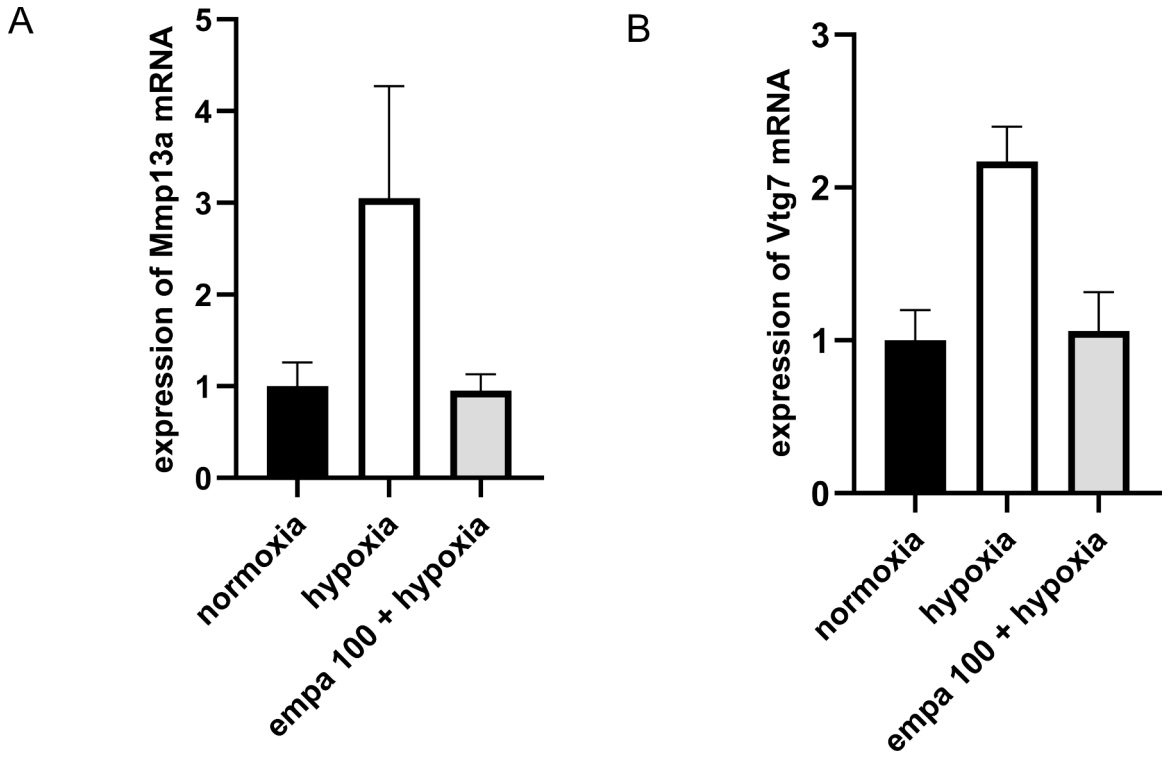

Fig. 3. Relative expression of Mmp13a (A) and Vtg7 (B) mRNAs derived from transcriptome analysis. Zebrafish embryos exposed to hypoxia were treated with empagliflozin (100 μM). Empagliflozin eliminated the increased expression of *mmp13a* and *vtg7* caused by hypoxia. Statistical analysis criteria used were: fold change > 2; false discovery rate < 0.05. Capped bars on top of the columns indicate standard deviations.

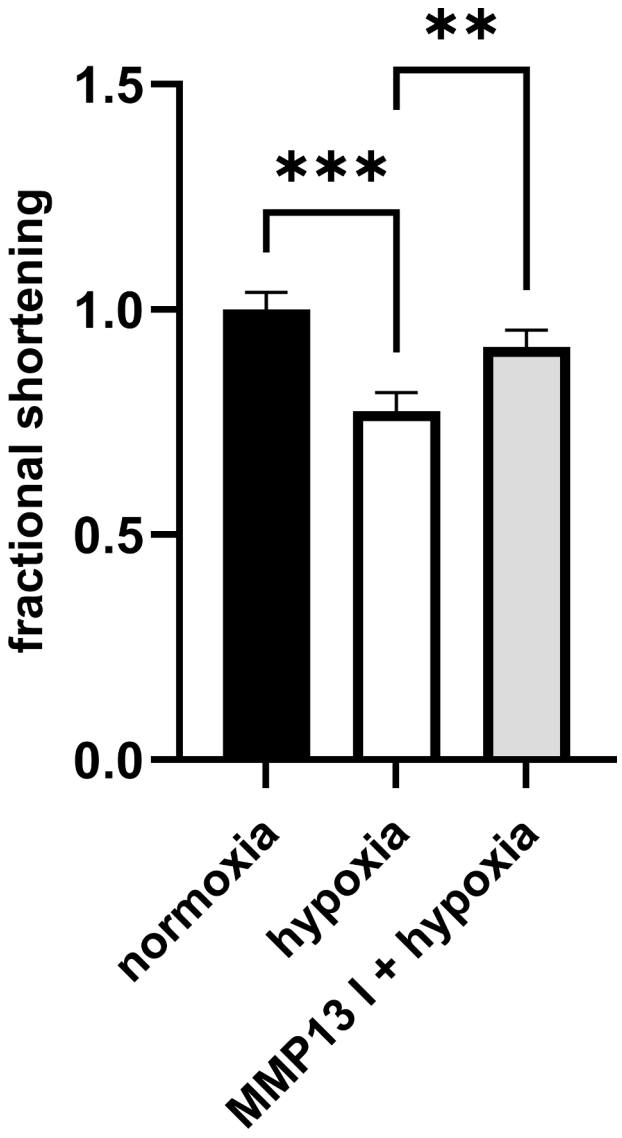

Fig. 4. Effect of MMP13 inhibitor (MMP13 I) on fractional shortening of hypoxia-exposed zebrafish embryos' hearts. The concentration of the MMP13 inhibitor was 40 μM. *** P<0.001, **P<0.01, *P<0.05. Capped bars on top of the columns indicate standard error of the means.

expression caused by hypoxia (Fig. 2. B).

3.2. Transcriptome analysis of total RNA derived from zebrafish embryos in normoxia and hypoxia, and the effect of empagliflozin on transcriptome profiles

To identify potential cardioprotective mechanisms responsible for the beneficial effect of empagliflozin on hypoxia-induced HF in zebrafish embryos, we carried out transcriptome analysis comparing total RNA expression profiles of zebrafish embryos subjected to normoxia and hypoxia. We found that 436 genes were differentially regulated in normoxia vs. hypoxia-induced HF model (Table 1). Of these 436 genes, 98 were upregulated and 338 were downregulated in response to hypoxia. Next, we compared the effect of empagliflozin on total RNA expression profile in response to hypoxia. The analysis demonstrated that in hypoxia-treated embryos empagliflozin influenced the expression of only two genes, namely the expression of *matrix metalloproteinase 13a* (*mmp13a*) and *vitellogenin7* (*vtg7*), both of which were downregulated in response to empagliflozin. (Table 1). The threshold value used in the statistical analysis was fc > 2 and the achieved false discovery rates (fdr) of zero indicate results of high significance [25]. Compared to normoxic conditions, exposure to hypoxia increased the expression of *mmp13a* and *vtg7* in zebrafish embryos 3.0- and 2.2-fold, respectively. Empagliflozin treatment was able to revert the decreased *mmp13a* expression caused by hypoxia nearly to the same level as the expression was in normoxia (Fig. 3. A). The results regarding *vtg7* expression were highly similar (Fig. 3. B).

3.3. Effect of MMP13 inhibitor on the heart function of zebrafish embryos in hypoxia

The above results suggested that the cardioprotective effect of empagliflozin in hypoxia is mediated via Mmp13a and Vtg7. However, because Vtg7 doesn't have an actual mammalian homologue, we focused only on Mmp13a to examine its role in hypoxia-induced HF. As empagliflozin reduced *mmp13a* expression, we aimed to test potential of pharmacological MMP13 inhibition in treatment of hypoxia-induced HF in zebrafish model. First, we tested the appropriate dose of the MMP13 inhibitor to be used in our zebrafish model. The results from these experiments showed that the MMP13 inhibitor was well-tolerated and did not impact the survival of embryos in any tested doses (Fig. S1). For the following experiments we chose the MMP13 inhibitor concentration of 40 μM. The results showed that MMP13 inhibitor was able to eliminate the hypoxia-induced decrease in FS of zebrafish embryo hearts (Fig. 4).

3.4. Effect of empagliflozin and MMP13 inhibitor on apoptosis and oxidative stress in the heart of zebrafish embryos in hypoxia

Ischemia-reperfusion of the heart may result in cell death and oxidative stress [26,27]. In zebrafish model the apoptosis has been analyzed using acridine orange (AO) staining [28], and we utilized this assay to analyze whether empagliflozin or MMP13 inhibitor effect on hypoxia-induced cell death in the heart of zebrafish embryos. The increased intensity of AO staining after hypoxia treatment indicated increased cell death. Interestingly, the AO signal in empagliflozin treated embryos subjected to hypoxia was significantly reduced whereas MMP13 inhibitor appeared to be ineffective (Fig. 5. A and B).

The cell death may result from exposure of the cells to reactive oxygen species (ROS) after reperfusion of the tissue, and reduced glutathione (GSH) is a key scavenger of ROS [29]. As monochlorobimane (mBCI) has been utilized as a GSH reporter in zebrafish [30], we stained zebrafish embryos after hypoxia-reperfusion with mBCI. Surprisingly, the mBCI signal did not decrease in hypoxia-treated samples as expected but instead a small increase in signal was observed. However, both empagliflozin and MMP13 inhibitor normalized this controversial response (Fig. 6. A and B).

4. Discussion

In the present study, we first demonstrated that empagliflozin is able to attenuate the HF of zebrafish embryos, in whom HF was caused by hypoxia. Specifically, we showed that empagliflozin normalized the decreased FS of embryos' hearts caused by hypoxia. In line with this, empagliflozin also normalized the increased *bnp* expression of the embryos in hypoxia. Our results are concordant with earlier studies with humans that have demonstrated a beneficial effect of empagliflozin on the heart function of patients with HF [7,31–33]. Our results confirm the general opinion on the zebrafish models that they provide invaluable *in vivo* models to evaluate the effects and also the potential mechanisms of therapeutics used in various cardiac diseases including HF [34].

Next, after demonstrating that empagliflozin is able to attenuate hypoxia-induced HF of zebrafish embryos we sought to examine potential mechanisms or actors playing a role in the beneficial effect of empagliflozin on the heart function of the embryos. For this purpose, we used transcriptome analysis. We found that there are two putative genes via which empagliflozin may act in attenuating the hypoxia-induced HF

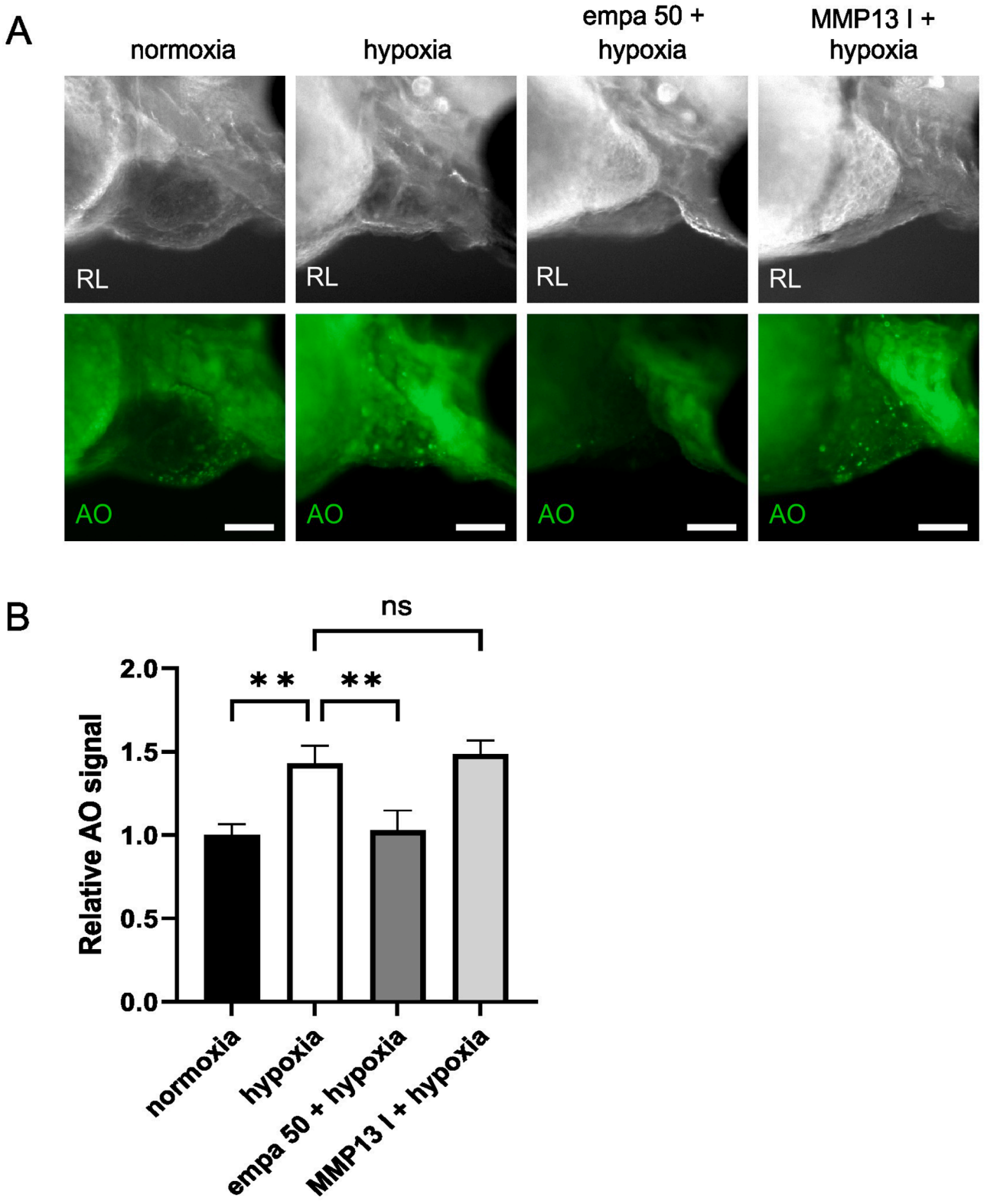

Fig. 5. Measurement of apoptosis in zebrafish embryo hearts using acridine orange. Embryos subjected to hypoxia and treated for 6 h with DMSO, empagliflozin (empa 50, 50 μM) or MMP13 inhibitor (MMP13 I, 40 μM) were stained with acridine orange (AO) and imaged with fluorescence microscope. A) Reflected white light (RL) and fluorescence images (AO) of zebrafish embryos. B) Quantitation of mean AO fluorescence intensity in the heart region. normoxia, n=11; hypoxia, n=9; empa 50 + hypoxia, n=7; MMP13 I + hypoxia, n=6. Scale bars 100 μm. **P<0.01, *P<0.05. Capped bars on top of the columns indicate standard error of the means.

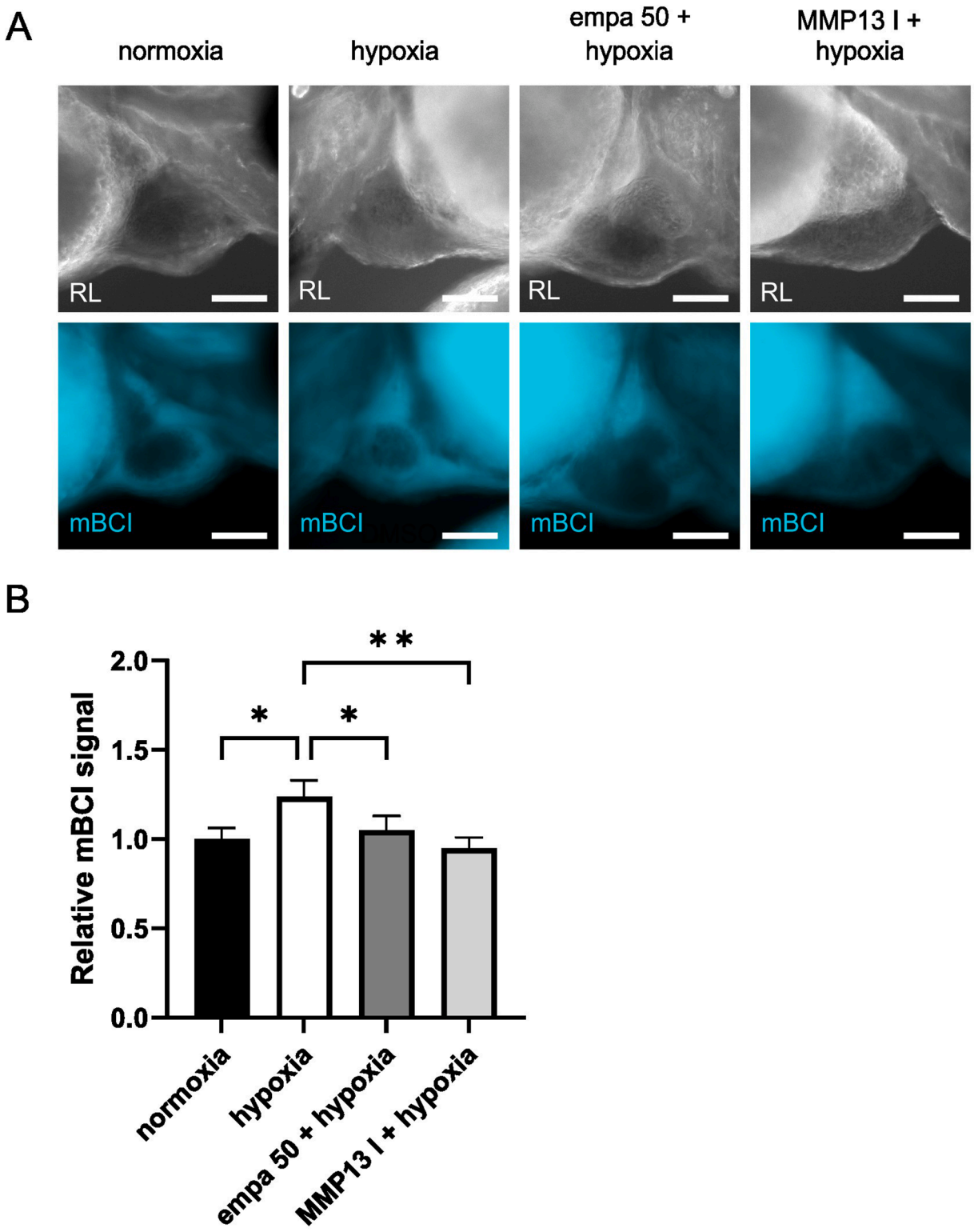

Fig. 6. Measurement of glutathione content in zebrafish embryo hearts using monochlorobimane.
Embryos subjected to hypoxia and treated for 24 h with DMSO, 50 μM empagliflozin (empa 50) or 40 μM MMP13 inhibitor (MMP13 I) were stained with monochlorobimane (mBCI) and imaged with fluorescence microscope. A) Reflected white light and fluorescence images of mBCI in zebrafish embryos. B) Quantitation of integrated mBCI fluorescence signal intensity in the heart region. normoxia, n=14; hypoxia, n=12; empa 50 + hypoxia, n=11; MMP13 I + hypoxia, n=13. Scale bars 100 μm. **P<0.01, *P<0.05. Capped bars on top of the columns indicate standard error of the means.

of the embryos. These two genes are *mmp13a and vtg7*, both of which were downregulated in response to empagliflozin (Fig. 3. A and B). Regarding vitellogenin, it is an ancient major yolk protein of eggs, where it is used as a food source during embryogenesis. Furthermore, it has been supposed to be evolutionarily homologous with parts of apolipoprotein B-100 of human low-density lipoprotein and human lipoprotein lipase [35]. Because Vtg7 doesnt have an actual mammalian homologue, we focused in this study only on Mmp13a. The rationale to focus on

Table 1
Number of differentially expressed genes (fc > 2 and fdr < 0.05) of zebrafish embryos after exposure to hypoxia and treatment with empagliflozin. For each treatment group (normoxia, hypoxia, hypoxia + 100 μM empagliflozin), n = 4.

Comparisons	Total	Upregulated	Downregulated
Hypoxia vs. normoxia	436	98	338
Empagliflozin 100 μM vs. hypoxia	2	0	2

Mmp13a was also based on the fact that MMP13 acts as a collagenase both in fish and mammals and it is a well-known fibrosis-associated factor contributing to several types of fibrosis including myocardial fibrosis [36–39]. On the other hand, hypoxia has been shown to induce MMP13 expression e.g., in nasopharyngeal cells and lungs [40,41]. Furthermore, empagliflozin [42–44] and other SGLT2 inhibitors, like canagliflozin and dapagliflozin, have been associated with the alleviation of cardiac fibrosis related events [45,46]. As such, we applied a specific MMP13 inhibitor in the experiments with zebrafish embryos in hypoxia. The results from these experiments clearly demonstrated that MMP13 inhibitor is able to normalize the harmful effect of hypoxia on the heart function of the embryos (Fig. 4). This result supports the idea that the beneficial effect of empagliflozin on the heart function of zebrafish embryos in hypoxia is, at least partially, mediated via MMP13. It is worth of noticing that there are no previous studies in any context demonstrating that empagliflozin has the ability to regulate MMP13 activity, although in a recent clinical study empagliflozin was shown to conduct anti-fibrotic effects on HF via regulating the levels of serum biomarkers of collagen turnover [47]. SGLT2 inhibitors have previously been shown to possess beneficial cardiac effects e.g., via inhibiting apoptosis [19]. Here, we have demonstrated that empagliflozin but not MMP13 inhibitor exhibits anti-apoptotic effect on the heart function on zebrafish embryos in hypoxia (Fig. 5), even if AO used in this study is somewhat unspecific for assaying apoptosis. Because also oxidative stress plays an important role in ischemia-reperfusion of the heart [26], we examined whether empagliflozin or MMP13 inhibitor might influence on the heart of zebrafish embryos in hypoxia via this mechanism of action using glutathione assay with monochlorobimane. Although, the expected decrease in the mBCI signal in hypoxia-treated samples was not observed but instead a small increase in signal was evident, empagliflozin and MMP13 inhibitor normalized this controversial response (Fig. 6). This indicates that they both influence the formation of reactive oxygen species in response to hypoxia. It is worth of noticing that empagliflozin has also been shown to significantly reduce infarct size in large animal models e.g., in a non-diabetic porcine model [48]. On the other hand, ischemia can create malignant arrhythmias leading to sudden cardiac deaths (SCD) [49]. Due to the fact that SGLT2 inhibitors are able to reduce SCD [50],the role of SGLT2 inhibitors in preventing arrhythmias remains to be clarified.

Finally, someone may criticize that due to the low expression of SGLT2 in the heart, it is difficult to interpret that the results from empagliflozin experiments are solely related with SGLT2 actions, because empagliflozin nearly exclusively binds to SGLT2 over to SGLT1, which is the dominating variant of the human cardiac SGLTs [51]. Therefore, it may be that empagliflozin as well as other SGLT2 inhibitors possess various pleiotropic effects on the heart that are not mediated via SGLT2. Indeed, this idea is supported by the results of Chen et al. [52] who recently showed that empagliflozin is able to reduce the infarct size in SGLT2-deficient mice.

5. Conclusions

In this study, we demonstrated using zebrafish embryos that empagliflozin is able to attenuate hypoxia-induced HF in zebrafish embryos via MMP13, which has earlier been shown to possess a causal role in HF. Our study provides a new potential mechanism whereby SGLT2 inhibitors exhibit a beneficial role in HF treatment. As such, our study supports the idea of developing MMP13 targeting new therapeutics for HF treatment in the future.

CRediT authorship contribution statement

Roope Huttunen: Writing – original draft, Investigation, Formal analysis. **Anna-Mari Haapanen-Saaristo**: Writing – original draft, Investigation, Formal analysis. **Ilkka Paatero**: Writing – original draft, Supervision, Project administration, Investigation, Formal analysis, Conceptualization. **Hannu Jarvelainen**: Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization. **Anja Hjelt**: Writing – original draft, Investigation, Formal analysis. **Anne Jokilammi**: Investigation, Formal analysis.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Hannu Jarvelainen reports financial support was provided by Satasairaala Central Hospital, The Wellbeing Services County of Satakunta Hannu Jarvelainen reports financial support was provided by Finnish Foundation for Cardiovascular Diseases. Hannu Jarvelainen reports financial support was provided by Turku University Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2024.117453.

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