Inhibition of the Sodium-Calcium Exchanger by SEA0400 Inhibits Manganese Efflux from Isolated Hearts

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Introduction

The sarcolemmal Na^{+}-Ca^{2+} exchanger (NCX) plays an important role in Ca^{2+} cycling in heart by regulating Ca^{2+} efflux during diastole. Recent studies indicate that manganese (Mn^{2+}) efflux occurs via NCX[1]. In the current study, we investigated whether manganese-enhanced MRI (MEMRI) with fast T_1 mapping allows sensitive detection of changes in NCX activity.

Methods

Heart Perfusion Protocol Male Sprague Dawley rats were anesthetized. The heart was excised, cannulated, and perfused with Krebs-Henseleit buffer equilibrated with 95% O_2 - 5% CO_2 at 37°C. A water-filled latex balloon was inserted into the left ventricle and connected to a pressure transducer to record the left ventricular pressure and heart rate. The rate-pressure product (RPP) was used as an index of workload. The perfusion column was placed in a vertical bore 9.4T Bruker scanner. Once the setup was finished, the heart was paced at 360 beats/min and the perfusate was switched to modified Krebs-Henseleit buffer containing 30 μM $MnCl_2$ for 30 min (washin period), followed by a 30 min washout period without $MnCl_2$. There were three experimental groups: 1) control: hearts perfused with Mn^{2+} only (n=5); 2) SEA0400_washin: hearts perfused with 1 μM SEA0400, an NCX inhibitor, during the washin period (n=6); and 3) SEA0400_washout: hearts perfused with 1 μM SEA0400 during the washout period (n=6).

MRI Study MR images were acquired with a 20 mm volume coil. A 1-mm thick short-axis slice at the midventricular level was prescribed for imaging. A triggered saturation recovery Look-Locker sequence was used for rapid T_1 mapping during Mn^{2+} perfusion and washout[2]. Pacing signals were used to trigger the image acquisition. Imaging parameters were: TE, 2 ms; TR, trigger interval (166 ms); flip angle, 10°; matrix size, 128x64; FOV, 2.5x2.5 cm^2. Prior to Mn^{2+} perfusion, two baseline T_1 maps were acquired. To delineate the kinetics of Mn^{2+} induced contrast enhancement, T_1 maps were acquired continuously at 3 min temporal resolution during the washin and washout period. Myocardial tissues were collected at the end of both washin and washout for ICP-AA analysis of Mn^{2+} content.

Results

Changes in relaxation rate (R_1) during the time course of perfusion are shown in Fig. 1. All three groups showed progressive increase in R_1 during the washin period. At the end of Mn^{2+} perfusion, R_1 in SEA0400_washin group was significantly higher than that in the other two groups ($p<0.001$). T_1 at the end of Mn^{2+} perfusion was 0.61±0.06 s, 0.51±0.05 s, and 0.59±0.03 s for control, SEA0400_washin, and SEA0400_washout groups, respectively (Fig. 2).

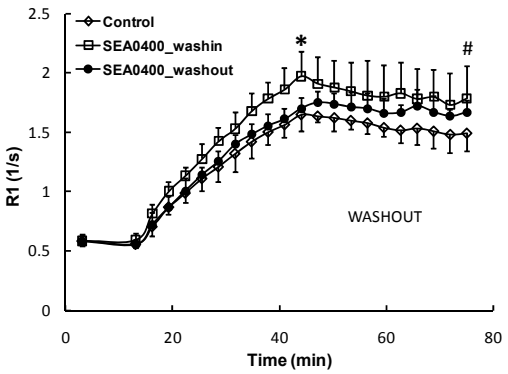

Figure 1. Dynamic changes in relaxation rate.

All three groups showed a slight decrease in R_1 during the washout period (Fig. 1). R_1 decrease was the smallest in SEA0400_washout group. Using linear regression, it was found that the slope of R_1 decrease was -0.0056 s^{-2}, -0.0047 s^{-2}, and -0.0031 s^{-2} for control, SEA0400_washin, and SEA0400_washout, respectively. At the end of washout, R_1 in both the SEA0400_washin and SEA0400_washout groups was significantly higher than that in the control group ($p<0.05$). T_1 at the end of washout was 0.68±0.07 s, 0.57±0.08 s, and 0.60±0.04 s for control, SEA0400_washin, and SEA0400_washout groups, respectively (Fig. 2).

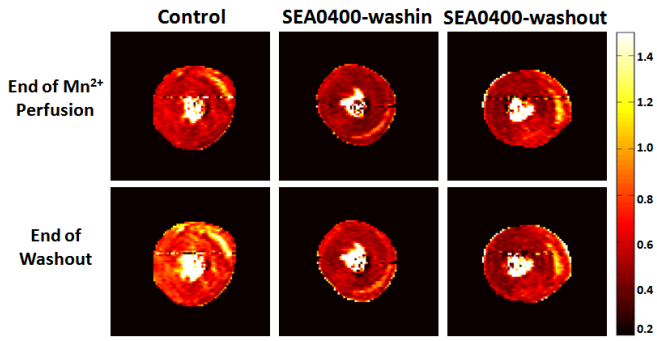

Figure 2. T1 maps at the end of washin and washout period.

NCX inhibition significantly increased Mn^{2+} content in SEA0400_washin hearts (12.9±1.6 μg/g wet weight) at the end of washin as compared to the controls (8.2±0.2 μg/g wet weight, $p<0.05$). Consistent with MRI findings, Mn^{2+} content at the end of washout was also higher in both SEA0400_washin (9.1±2.5 μg/g wet weight) and SEA0400_washout (9.5±3.0 μg/g wet weight) as compared to the controls (6.3±0.9 μg/g wet weight, $p<0.05$). Ventricular function remained constant during image acquisition in the control group, while SEA0400 perfusion induced a small increase in RPP in both SEA0400_washin and SEA0400_washout groups.

Conclusion

NCX inhibition via SEA0400 increased Mn^{2+} retention in the myocardium, leading to greater R_1 increase during washin period and less R_1 reduction during the washout. Our results suggest that MEMRI is also sensitive to changes in NCX activity.

Reference
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2. Wen L. et al., MRM **64**:1296-1303,2010.