GSTM2-2 C terminus modulates the contractility of cultured rat ventricular cardiomyocytes

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The ryanodine receptor (RyR) functions as an ion channel that releases Ca^{2+} from the sarcoplasmic reticulum and is essential for excitation-contraction coupling and contraction in striated muscle. In previous studies we have shown that the human muscle specific glutathione transferase M2-2 (GSTM2-2) is a high affinity inhibitor of cardiac muscle ryanodine receptors (RyR2) and a weak activator of skeletal muscle ryanodine receptors (RyR1) (Abdellatif *et al.*, 2007). Excessively active RyR channels are partly responsible for low store Ca^{2+} levels and defective release in heart failure. Therefore, inhibition of RyR2 is a potential strategy for the treatment of heart failure. GSTM2-2 is one of the few selective inhibitors of RyR2 that does not influence skeletal muscle RyR activity. Single channel lipid bilayer experiments and Ca^{2+} release assays conducted on the C-terminal half of GSTM2-2(GSTM2C) and the mutants, F157A and Y160A in the C terminal domain confirmed the importance of the helix 6 in the C-terminal fold for the inhibition of RyR2 (Hewawasam *et a*l., 2010). It has been reported in a qualitative study that a glutathione transferase from *Schistosoma japonicum* (Sj.GST26) can be internalised from the medium into a variety of mammalian cell types (Namiki *et al.*, 2003). Morris *et al.* (2008) also reported that proteins including GSTM2-2 containing a GST fold structure are efficiently internalized by L-929 cells. Therefore, the objective of this study was to confirm the fact that GSTM2C is capable of internalising into cultured cardiomyocytes and to determine the effect of GSTM2C on the cardiac function.

The study was performed on primary cardiomyocyte cultures from neonatal rats. Cells were seeded at a density of 1×10^5 cells per 35mm dish and used 2-3 days after plating. 1μM GSTM2C tagged with oregon green dye was incubated with cultured cardiomyocytes for 24h and immunostained with anti α-actinin, which specifically stains α cardiac/skeletal actinin. Optical fields were randomly chosen and observed to ascertain the occurrence of spontaneous beats. Beating frequency and number of beating cells were counted from the control and 1μM GSTM2C treated cells. Cell beating was recorded in both control and GSTM2C treated cells using a JVC video camera KY/F550 attached to Nikon TE2000-U microscope. Images were analysed using Image Pro plus 6.2 software and percentage cell shortening measured.

Confocal images of the cardiomyocytes stained with Oregon green-GSTM2C and anti α-actinin confirmed the uptake of GSTM2C into the cultured cardiomyocytes. A preliminary study showed that the beating frequency (contractions per min) in the control group was reduced significantly from 42.5/min to 6.9/min in the GSTM2C treated cells ($p<0.001$). The number of spontaneously beating cells observed in the control group, 6.6%, was also significantly reduced to 1.9% ($p<0.001$) in the drug treated group. In order to determine whether the above result is due to action potential failure or GSTM2 C terminus affecting the contraction mechanism, the degree of shortening in each beat was measured. The percentage shortening was significantly reduced from 7.5±1.0% in the control group of cardiomyocytes to 2.9±0.6% after GSTM2C treatment ($p<0.001$), consistent with GSTM2C reducing Ca^{2+} release from the SR.

In conclusion, our results indicate that GSTM2 C terminus enters cardiomyocytes and alters the cardiac function by reducing Ca^{2+} release through RyR2 in ventricular cardiomyocytes.

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