

Further investigation into the cardiovascular safety profile of muscarinic antagonist, ipratropium

Katherine Simmons, Afthab Hussain, Helen Maddock. Coventry University, CV1 5FB, United Kingdom.

Ipratropium Bromide, a non-selective muscarinic receptor antagonist, is frequently prescribed for the management of Chronic Obstructive Pulmonary Disease (COPD). However, conflicting issues relating to the cardiac safety of ipratropium have been raised. These include an indicated increased risk of myocardial infarction in COPD patients (Ogale et al., 2010). Previous work in our laboratory using a model of myocardial ischaemia/reperfusion revealed that ipratropium exacerbates myocardial injury under these conditions.

The aims of the project were to further determine the specific involvement of ipratropium at the muscarinic receptor on myocardial ischaemia/reperfusion injury in a whole heart Langendorff model and isolated adult cardiomyocyte model of oxidative stress.

Isolated hearts were subjected to 35 minutes of ischaemia and 120 minutes of reperfusion in the presence and absence of ipratropium (0.01 and 0.1 μ M, n=7) and / or acetylcholine (muscarinic receptor agonist, 0.1 μ M). At the end of the experiment hearts were stained with Evans blue and incubated with 2,3,5-triphenyl tetrazolium chloride to determine the infarct size to risk ratio (%). Administration of ipratropium throughout reperfusion significantly increased infarct size to risk ratio (%) compared with non-treated controls (62 \pm 2% and 74 \pm 4% vs. 52 \pm 3% Control P<0.01 respectively).

To determine whether the toxic effects of ipratropium were due to a specific action on the mitochondria, the time (seconds) for mitochondrial depolarisation (Dep) (the loss of mitochondrial membrane potential) and Hypercontracture (Hyp) (subsequent cell death) to occur were recorded via use of confocal microscopy. Isolated adult cardiac myocytes were subjected to oxidative stress in the presence and absence of ipratropium (0.01, 0.1 and 1 μ M) and acetylcholine (0.1 μ M). Ipratropium significantly reduced the time taken for Dep and Hyp of the cardiac myocytes (Table 1, n=3). Acetylcholine, when administered in the presence of ipratropium, significantly reversed the toxic effects of ipratropium. This is the first study to show that the exacerbation of myocardial ischaemia/reperfusion injury by ipratropium involves disturbance of the mitochondria.

Table 1: Effect of ipratropium in the presence and absence of acetylcholine on mitochondrial membrane potential and hypercontracture of adult myocytes

Adult Cells	Control	Ipratropium (0.1 μ M)	Acetyl-choline (0.1 μ M)	Ipratropium+ Acetyl -choline	FCCP
Depolarization	206 \pm 10	148 \pm 18 *	277 \pm 26*	237 \pm 9 **	11 \pm 4 *, **, #
Hypercontracture	550 \pm 12	455 \pm 15 *	573 \pm 23	548 \pm 12 **	136 \pm 23 *, **, #

*p<0.05 vs. Control, **p<0.01 vs. Ipratropium