Mitochondrial toxicity of Metformin and Phenformin assessed by respirometry of human peripheral blood cells

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Purpose: Metformin and Phenformin are anti-diabetic drugs. Phenformin has been banned from the market due to the high incidence of lactic acidosis (LA).[1] Metformin is generally considered as safe but the development of LA has been reported in rare cases.[2] In both cases, LA is thought to be linked to drug-induced mitochondrial dysfunction in the liver and other tissues.[1] Using high-resolution respirometry it was investigated whether Metformin- and Phenformin-induced mitochondrial toxicity can be detected in human peripheral blood cells.

Methods: Thrombocytes were isolated according to Sjövall *et al*[3] and white blood cells were isolated by Ficoll gradient centrifugation[4]. The integrated function of mitochondria in both intact and permeabilized blood cells was studied using high-resolution respirometry. The cells were treated with a wide concentration range of Metformin, Phenformin or vehicle to assess direct effects by the compounds on respiration. Thereto the time-dependent effect of Metformin on endogenous respiration in intact thrombocytes was followed for 60 minutes. In permeabilized cells, a multiple substrate-uncoupler-inhibitor-titration (SUIT) protocol was used in order to determine maximal respiratory capacities and the site of toxicity of Metformin and Phenformin.

Results: Intact thrombocytes showed a decreasing routine respiration and maximal electron transfer system (ETS) capacity with increasing doses of Metformin compared to controls. In permeabilized thrombocytes, Metformin and Phenformin induced a dose-dependent reduction of maximal oxidative phosphorylation and uncoupled ETS capacity predominantly through inhibition of complex I. Lower concentrations of the drugs were required to induce respiratory inhibition in permeabilized cells compared to intact, but the toxic effect in intact cells developed over time. Although a qualitatively similar toxic effect was seen for both drug treatments, the cells were more sensitive to treatment with Phenformin than Metformin when using the same concentrations. The toxic effect of Metformin was also similar in permeabilized white blood cells.

Conclusion: Metformin and Phenformin have a direct toxic effect on mitochondrial function in human peripheral blood cells. The reduction of respiration is predominantly mediated through a dose-dependent inhibition of complex I. Respirometry of peripheral blood cells may be a suitable assay to predict mitochondrial toxicity of drugs in humans.

References:

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