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A comprehensive investigation of copper binding properties of metformin using on-disc magnetic microbead agglomeration with real-time analysis

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Introduction: Metformin is a widely used type-2 diabetes drug. Its copper-binding properties are known to be crucial but still not fully understood. We present a comprehensive investigation of its interaction with L-cysteine-Cu complex using a magnetic microbead (MB)-based assay on a microfluidic disc. The assay scheme is similar to the one presented in our previous study, where optomagnetic readout and magnetic nanobeads were used [1]. In this study, we have significantly simplified the detection by using an optical scanning method [2] and micrometer-sized beads. Additionally, we can measure the effect of 100-fold lower concentration of metformin than in our previous study. Our results clearly illustrate the strong metformin-Cu interaction and provide the opportunity of real-time analysis.

Methods:

The assay platform is a microfluidic disc made of Poly(methylmetacrylate) (PMMA) bonded together using pressure-sensitive adhesive (Fig.2a). For studying L-cysteine-Cu interaction, Cu solution and 1µm streptavidin-coated MBs (1mg/ml) functionalized with biotinylated L-cysteine (volume ratio 1:11) were incubated for 10 minutes with gentle shaking and then loaded into the disc. For studying interaction between metformin and L-cysteine-Cu complex, metformin was added to a separately pre-incubated L-cysteine-Cu (volume ratio 2:1) solution, further incubated for 5 minutes and loaded into the disc. Finally, all samples were incubated on-disc under permanent magnetic field with continuous shaking for 10 minutes before scanning by oCelloScope (Fig.2b).

Results: Scanning results of four different samples are illustrated in Fig.3.
We performed real-time analysis of cluster formation as illustrated in Fig4. 

**Fig3:** (a) Magnetic beads functionalized with biotinylated L-cysteine used as the control sample (b) L-cysteine functionalized MBs incubated in Cu solution followed by 10 min magnetic incubation. MB clusters form because of the formation of L-cysteine-Cu complex. (c) & (d) After the addition of 2.1 µM and 21 µM metformin into two separate mixtures of Cu and L-cysteine functionalized MB followed by magnetic incubation, the MB-clusters are broken in respective order for the breakage of L-cysteine-Cu complex and formation of metformin-Cu complex. (e) Measured MB cluster size vs amount of metformin in the sample: we conclude that samples with 21 µM and 2.1 µM metformin have 57.9% and 34.6% reduced mean cluster size respectively than the sample with only Cu. Scale bar: 50 µm.

**Fig4:** L-cysteine coupled MBs were incubated in Cu$^{2+}$ solution for 15 minutes, which resulted in an insignificant visible change in size of particles. Magnetic incubation for 10 mins resulted in the formation of large clusters (mean area: 233.68 µm²) which shows the significance of an optimized magnetic incubation. Next, 5 mins incubation with metformin followed by 10 min magnetic incubation showed MB clusters of about 94.24 µm² mean area i.e. addition of metformin caused 40% averaged reduction of the L-cysteine-Cu-MB cluster size. Additional incubation of 15 mins was performed showing no significant changes.

**Discussion:** The presented results show that metformin, even at low concentration, is able to break the L-cysteine-Cu complex, which can affect mitochondrial function [3]. The investigation provides in-depth insight on the metformin-Cu interactions by quantifying the size of the clusters formed by molecular reaction. Thus, the novel detection strategy and kinetics analysis could be used for studying metal-binding properties of metformin-analogues.

**References:**