Potential of extracellular vesicles as surrogates for the cellular ABC transporter expression

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INTRODUCTION AND AIM

Extracellular vesicles (EVs) are nanoparticles released by all types of cells and comprise exosomes and microvesicles. EVs contain, among other proteins, ABC-transporters, the most relevant drug-efflux pumps (Fig.1). Studies demonstrated that ABC-transporters are differentially expressed within the population, and in this regard, their quantification in EVs could be a promising tool to personalise pharmacotherapy[1,2].

The project aims to evaluate the suitability of EVs as a surrogate for cellular ABC–transporter expression *in vitro* (Fig. 2).

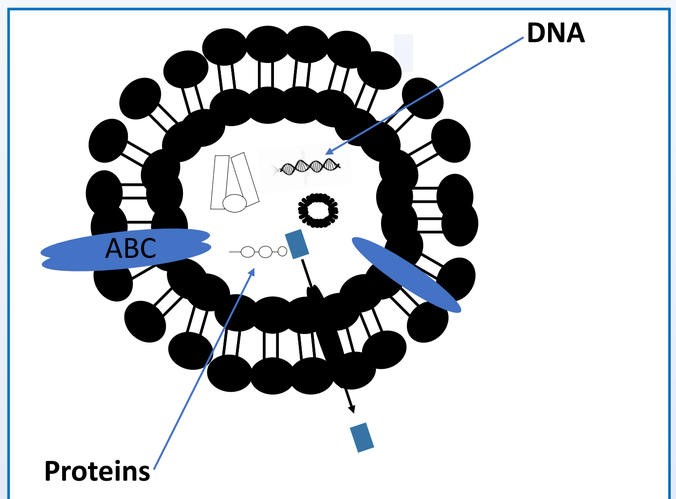

Fig 1. Extracellular vesicle composition

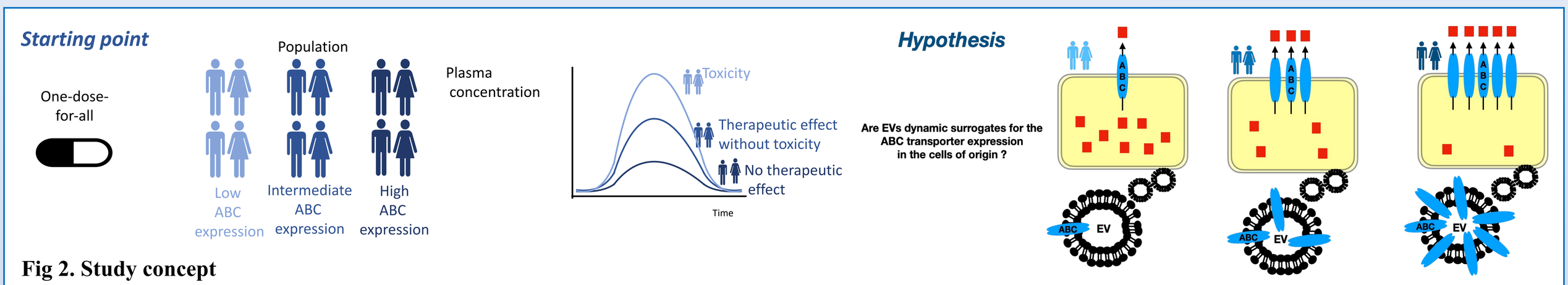

Fig 2. Study concept

METHODS

MDCK (i.e., canine kidney) cells with overexpression of human P-glycoprotein (Mdr1/P-gp/ABCB1), breast cancer resistance protein (BCRP/ABCG2) or multidrug-resistance associated protein (MRP2/ABCC2) were used in the first experiments[3]. EV isolation has been performed from cell culture supernatant through various steps, including ultracentrifugation and filtration[4]. Confirmation of proper isolation has been achieved with dynamic light scattering method (DLS, Zetasizer NanoZS device). Quantification of P-gp, MRP2 and BCRP has been conducted using a UPLC-MS/MS system (Xevo TQ-XS).

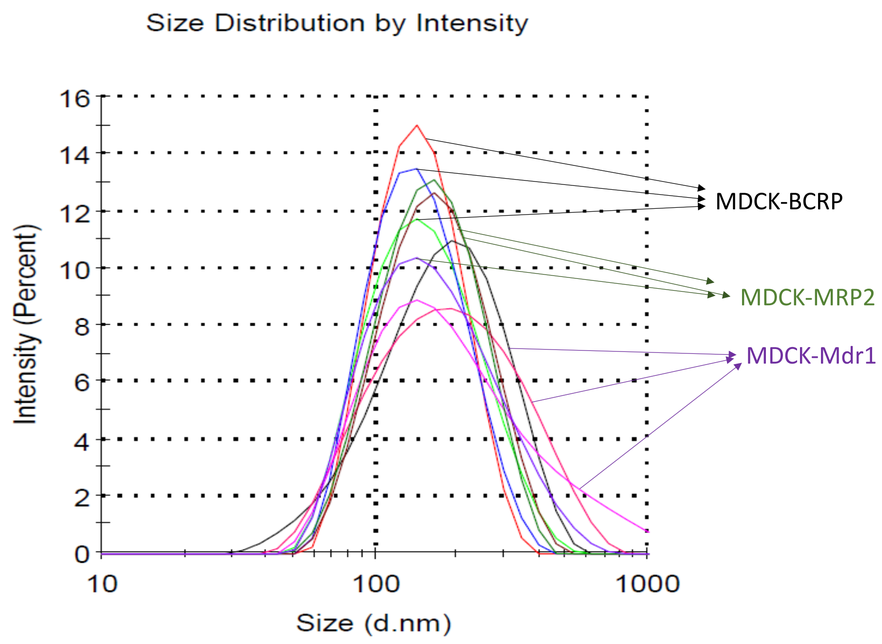

Fig 3. Analysis of EV fractions from MDCK-Mdr1, MDCK-MRP2 and MDCK-BCRP cells by DLS.

RESULTS

Our analysis showed that the size of the EVs, as determined by DLS, was 172.2 ± 3.5 nm, 157.8 ± 2.7 nm and 143.1 ± 1.5 nm, for EVs derived from MDCK-Mdr1, MDCK-MRP2 and MDCK-BCRP cells, respectively, compatible with an exosome-enriched EV sample (Fig. 3).

Then, the simultaneous detection of three surrogate peptides (for P-gp/Mdr1, MRP2, and BCRP) designed to quantify ABC transporter expression by UPLC-MS/MS was assessed. Higher MRP2 levels were observed in MDCK-MRP2-derived EVs respect to MDCK cells without MRP2 overexpression, providing preliminary evidence of an association between the expression of this transporter in EVs and in the cells of origin.

On the contrary, no association was observed for P-gp or BCRP (Fig. 4).

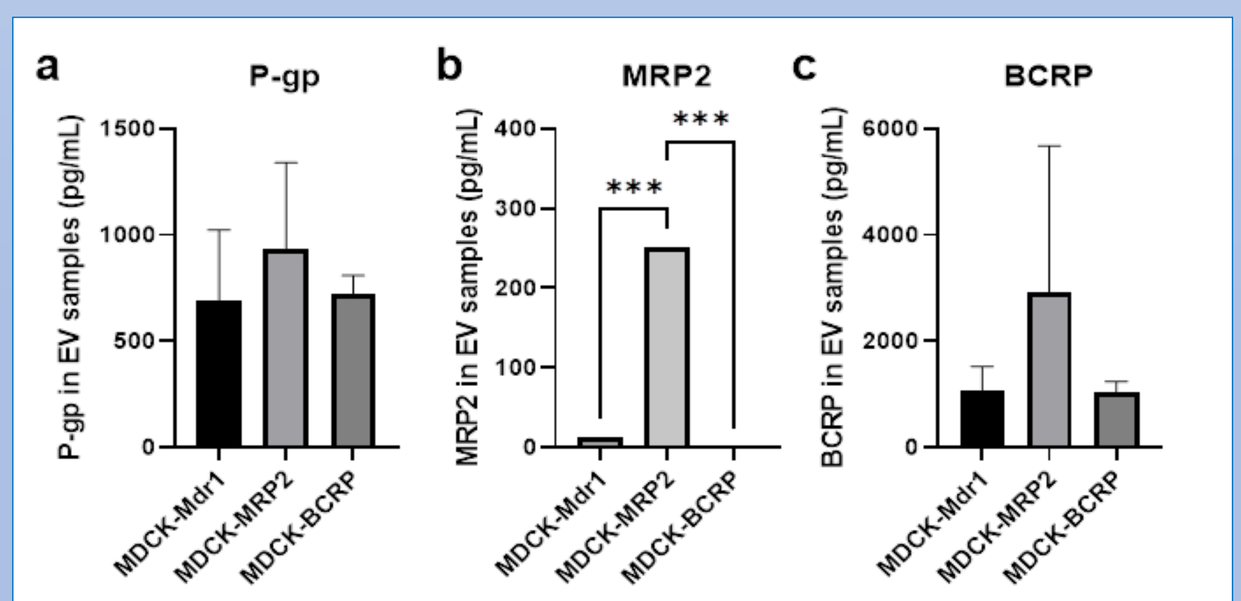

Fig 4. UPLC-MS/MS quantification of P-gp (a), MRP2 (b) and BCRP (c) in EVs derived from MDCK-Mdr1, MDCK-MRP2 and MDCK-BCRP cells (c). * $p<0.005$, n=3.

DISCUSSION

First results point to the potential of EVs as surrogates for the expression of MRP2 in the cells of origin. In the near future, transporter expression will be determined in EVs and the corresponding human cell lines under basal conditions and after up-regulation with the inducer rifampicin (e.g., 0.1-20 μM).

In the era of precision medicine, this field can be particularly promising, giving that EVs are present in all biological fluids and readily accessible via liquid biopsies, not only to diagnose diseases but also to predict drug disposition, efficacy and toxicity

References

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