Cryopreserved human hepatocytes – optimization of uptake assays

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Abstract

Cryopreserved human hepatocytes may be used as an *in vitro* model to assess transporter uptake activity by the liver. Hepatocytes in suspension are incubated with (radiolabeled) substrates and a test article (TA) to assess drug drug interactions (DDIs), or with a TA alone to assess uptake potential. Cells may be separated from the assay buffer by the traditional oil spin method[1], after which the amount of substrate or TA present in the cells is measured.

The principle of the traditional oil spin method relies on separation of cells from suspension by rapid centrifugation of the cells through a layer of oil. As an alternative the filter plate method that relies on separation of cells from suspension by vacuum filtration, can be used,.

We used tritiated substrates to compare both methods: taurocholate (TC) for NTCP, methyl-4-phenylpyridinium (MPP+) for OCTs and estrone-3-sulfate (E3S) as well as estradiol 17-beta glucuronide (E2-17bG) for OATPs.

Results are reproducible by both methods and correlate well with previously reported results[2]. The standard deviation (n=3) is low, for all substrates. Fold activations are equal between methods. For E3S, TC and E2-17bG the oil spin method yields in higher detection of uptake, whereas more MPP+ is detected with the filter plate method.

Objective

To validate the use of the filter plate method by comparing transport rate and fold activation with the oil spin method.

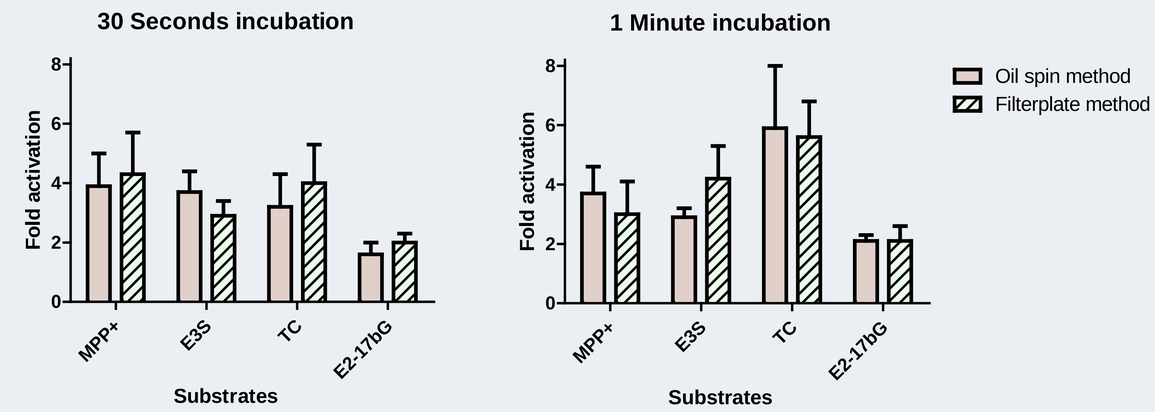

Figure 2 Average fold activation (active transport divided by passive uptake) of different lots for probe substrates MPP+ (1µM), E3S (2 µM), TC (1 µM) and E2-17bG (0.1 µM) assesed at 30 seconds or 1 minute of incubation in both oil spin and filter plate method. Error bars represent the standard deviation (n=3).

Materials and Methods

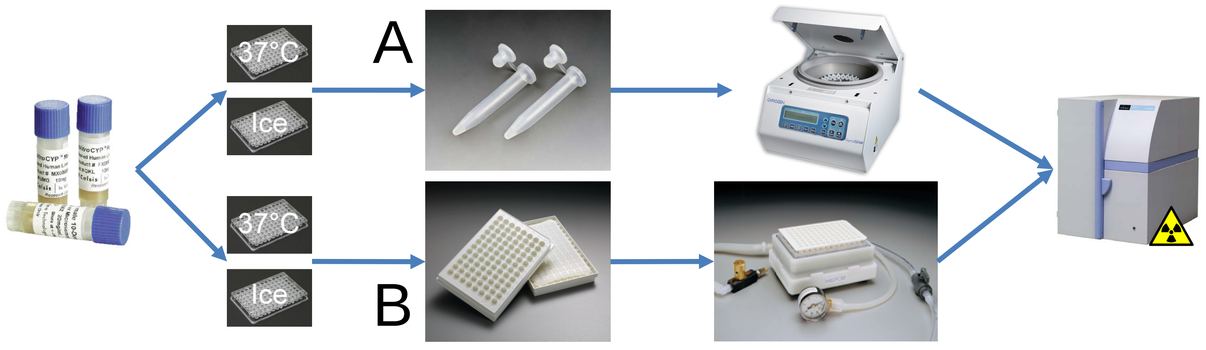

Figure 1 Two vials per lot of cryopreserved human hepatocytes were thawed, pooled, distributed in 96-wells plates (n=3) and incubated with prototypical substrates at 37°C or on ice for 30 seconds or 1 minute.
A) Oil spin method: Upon approaching the incubation time, the cells were layered on top an oil layer in a prepared tube containing a 2N sodium hydroxide solution at the bottom. At the desired incubation time the tubes were centrifuged at high speed by which the hepatocytes migrated through the oil into the alkaline solution. The lower part of the tubes was cut off and subjected to liquid scintillation counting.
B) Filter plate method: Upon reaching the incubation time, cold washing buffer was added and the cells were transferred to a pre-wetted filter plate. Vacuum was applied immediately and cells were washed 5 times with cold buffer. The filter plate was dried and subjected to liquid scintillation counting.

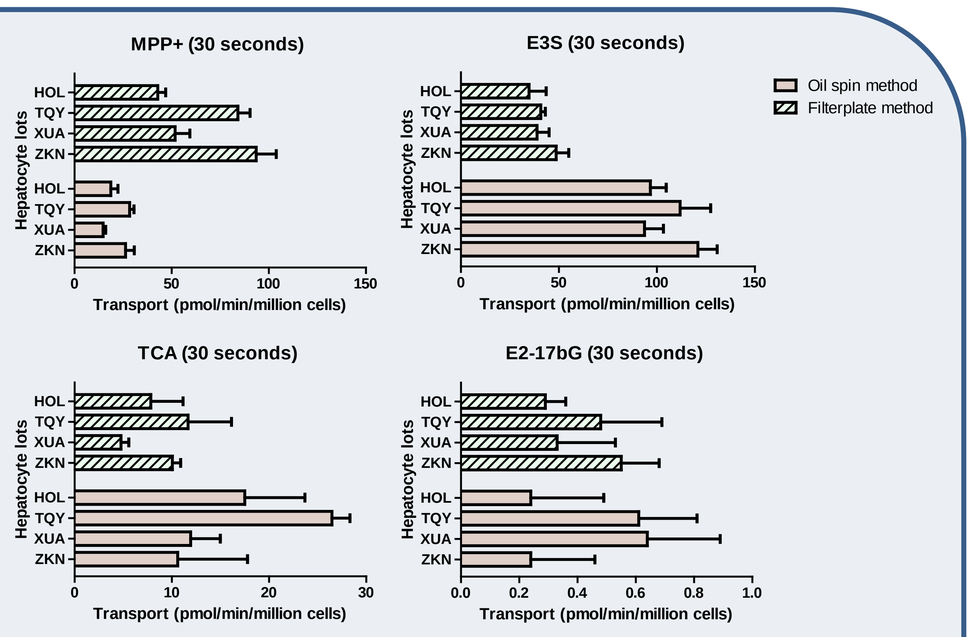

Figure 3 Uptake of prototypical substrates (MPP+ (1µM), E3S (2 µM), TC (1 µM) and E2-17bG (0.1 µM)) in different lots of cryopreserved hepatocytes assayed by the oil spin and filter plate method. Error bars represent the standard deviation (n=3).

Results

The fold activation data (active transport {37°C} divided by passive uptake {4°C}) after 30 seconds of incubation shows that a general of 3.5 - 4 fold activation is obtained for substrates MPP+ and TC, whereas E3S shows a 3 fold activation and E2-17bG 2 fold. After 1 minute of incubation the same numbers are observed, with the exception of TC, which shows an average of approximal 5.5 fold difference (Figure 2).

Higher E3S, TC and E2-17bG accumulation (2-3 times) is detected when using the oil spin method in direct comparison for all lots and independent of incubation time. On the contrary, higher MPP+ accumulation (3 times) is detected when the filter plate method was used (Figure 3 and 4).

The rate of transport (pmol/min/millions cells) for all compounds is slightly higher at 30 seconds of incubation than at 1 minute of incubation with fold differences for the oil and filter plate method of 1.8 and 2 (MPP+), 1.5 and 1.4 (E3S), 1 and 1.5 (TC) and 1 and 2.3 (E2-17bG), respectively.

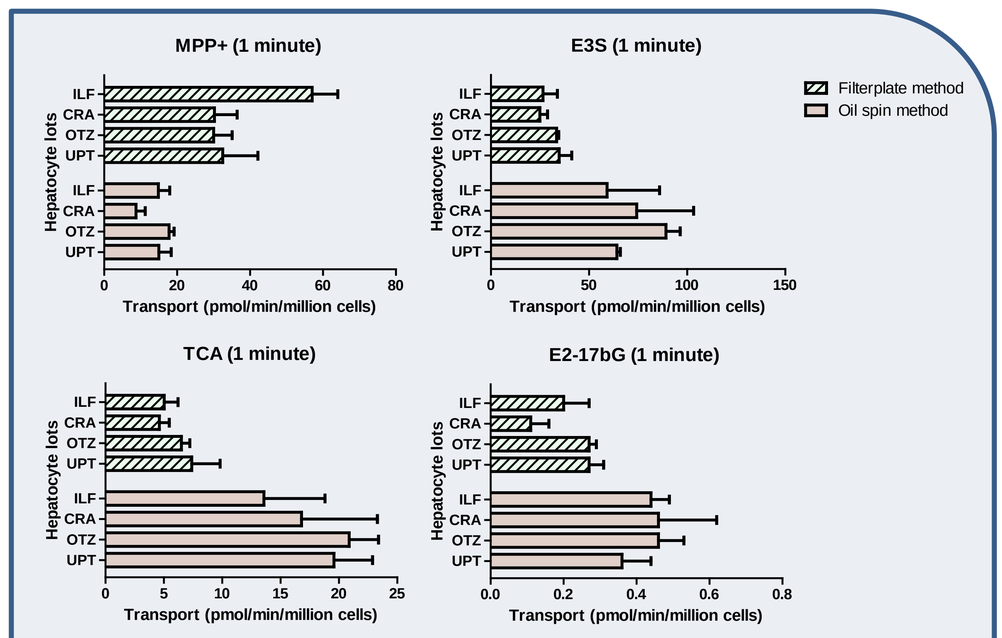

Figure 4 As in Figure 3 but different lots of hepatocytes and different incubation times. Error bars represent the standard deviation (n=3).

Conclusions

The oil spin method yields higher transport rates for all tested compounds, with the exception of MPP+.

With regard to fold activation and standard deviations both methods score equally well, indicating that both methods are suitable for conducting relative interaction studies such as an IC50 determination.

The filter plate method can be prefered over the oil spin method, because time can be saved and higher througput of samples is possible.

References
1) Y. Shitara *et al.*, Drug Metab- Pharmacokin. 18 (1):33-41 (2003)
2) T. De Bruyn *et al.*, Europ. J. of Pharmaceut. Sc. 43 297–307 (2011)