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Establishing a platform of uptake transporters in HEK-293 cells for the analysis of possible drug–drug interactions

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Membrane transporters are major variables for disposition, efficacy and safety of drugs. Organic anion transporting polypeptides (OATPs, *SLCO*), Na^+-taurocholate co-transporting polypeptide (NTCP, *SLC10A1*), organic cation transporters (OCT, *SLC22*) and organic anion transporters (OAT, *SLC22*) belong to the uptake transporters and mediate the uptake of a broad range of substrates including several widely prescribed drugs. We have established a cell platform using stably transfected cells expressing pharmacologic relevant uptake transporters to analyze drug affinities. Here, the transporter activities are analyzed with fluorescent substances in assays that can be performed in standard laboratories. Transporter activities of OATP1A2, -1B1, -1B3, -2B1 and NTCP as well as OCT1, -2, -3 and OAT2, -3 were analyzed with five fluorescent substances (fluorescein methotrexate (FMTX), fluorescein, rhodamine 123, dibromofluorescein (DBF), cholyl-lysyl-fluorescein (CLF)). FMTX was specifically transported by OATP1B1, -1B3 and OAT2, -3. Fluorescein is a substrate for OATP1B1 and OAT2, -3, while rhodamine is one for HEK-OATP1A2 and OCT1, -2, -3. CLF was characterized as substrate for NTCP, OATP1B1 and -1B3. DBF is transported by HEK-OATP2B1. Rifampicin functions as inhibitor for all investigated OATPs. Transport activity of all 3 OCTs could be inhibited by quinidine. Cholate efficiently inhibited the uptake of CLF mediated by NTCP. Diclofenac functions as inhibitor for OAT2 and OAT3. Transport function of stably transfected HEK-293 cells expressing the above mentioned transporters could be analysed with fluorescent substances. This platform can be used for identification of specific transporters involved in drug uptake and drug–drug interactions.

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Elimination effect of haemoperfusion on different initial level of plasma paraquat concentration in vivo and in vitro

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Paraquat poisoning is frequently fatal and haemoperfusion as a key therapy has been widely used. This study was to estimate the PQ clearance of current HP protocol on different initial PQ concentration. This study comprised two parts and approved by IRB: in vivo evaluation with acute PQ poisoning patients. 35 patients were divided into three groups according to the initial plasma PQ concentration using Mass Spectrum. In vitro investigation of heparin treated whole blood with three different PQ dose preinfused. HP with similar clear capacity (ml/min/g) were performed both in vivo and in vitro by reducing resin weight and flow in proportion to the blood volume. The plasma samples were obtained in 1 h and 2 h after HP for the reduction rate analysis. The PQ reduction rate was slightly different in vivo than in vitro. The PQ clearance decreased rapidly after one hour in all groups, especially in vitro high dose group ($P<0.01$). In vitro, the total PQ reduction rate of each group was $54.33 \pm 15.24\%$, $86.35 \pm 4.56\%$, $72.82 \pm 10.29\%$, respectively. While in vivo, was $41.92 \pm 33.43\%$, $85.14 \pm 7.88\%$, $60.14 \pm 24.19\%$, respectively. The PQ clearance of HP was significantly greater in moderate dose both in vivo and in vitro ($P<0.01$). The PQ clearance effect of current HP protocol was significant different in terms of different initial PQ level. Adequate HP protocol adjust with PQ concentration appears to be an indispensable treatment for patients with acute PQ poisoning.

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Effect of inhalation exposure to toluene in Oatp activity using pravastatin as a probe drug in rats

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Organic anion transporting polypeptides (OATP in humans, Oatp in rats) are drug transporters expressed in hepatocytes and its activity has been associated to the hepatic uptake and clearance of statins. The influence of occupational exposure to chemicals on the variability in drug response is little explored in the literature. The objective of this study was to evaluate the influence of inhalation exposure to toluene in Oatp activity using pravastatin as a probe drug in rats. Male Wistar rats ($n=6$, for each sampling time) were exposed to 85 mg/m^3 toluene by inhalation or air in a nose only exposure system for 6 h/day, 5 days/week during 4 weeks, in order to simulate the occupational exposure to toluene at level slightly above the occupational exposure limit proposed by ACGIH. After 4 weeks of exposure, animals received a single dose of 20 mg/kg pravastatin orally (gavage). Pravastatin plasma concentrations were analysed by LC–MS. Areas under concentration × time curves extrapolated to infinite ($AUC^{0-\infty}$) were calculated by Gauss Laguerre quadrature. Non-exposed animals showed $AUC^{0-\infty}$ (mean ± relative standard error) of 726.0 ± 261.8 ng h/mL for pravastatin. No significant difference was observed in $AUC^{0-\infty}$ (681.8 ± 80.1 ng h/mL) when rats exposed to toluene were compared to non-exposed rats. Toluene exposure by inhalation did not change the in vivo activity of Oatp evaluated by pravastatin kinetic disposition in rats.

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