Supplementary Material

Sorafenib activity and disposition in liver cancer does not depend on organic cation transporter 1 (OCT1)

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Supplementary Methods

Chemicals and reagents

General tritium-labeled sorafenib and sorafenib-N-oxide (specific activity, >37 GBq/mmol; radiochemical purity, >97.1%) were purchased from Moravek. The positive control substrates [^{14}C]tetraethylammonium bromide (TEA; specific activity, 130 MBq/mmol) and [^{3}H]1-methyl-4-phenylpyridinium ([^{3}H]MPP, 2.96 TBq/mmol) were obtained from PerkinElmer and American Radiolabeled Chemicals Inc., respectively. Chemicals were obtained at the highest purity available from various commercial sources.

Transport studies using OCT1-expressing oocytes

OCT1-expressing oocytes of *Xenopus laevis* were generated as described[1] using 0.1 µg/µl of human OCT1/SLC22A1 cRNA for injection. Oocytes were then stored for 2 days in Ori buffer at 16°C. For transport measurements, OCT1-expressing oocytes and non-injected control oocytes were incubated at room temperature for 20 minutes in Ori buffer as described[1] containing 12 nmol/l [^{3}H]MPP or 1 µmol/l [^{3}H]sorafenib in the absence or presence of the OCT1 substrate and inhibitor MPP. The oocytes were then washed 3 times in ice-cold Ori buffer, solubilized in 5% sodium dodecyl sulfate (SDS), and intracellular radioactivity was analyzed by liquid scintillation counting (Packard TriCarb 1600; PerkinElmer, Waltham, MA, USA).

Mammalian cell culture and immunolabeling of cells

HepG2, HEK293 and Huh7 cells were maintained in DMEM medium (Sigma) and HeLa cells in RPMI-1640 medium (Biochrom) with 10% fetal calf serum (FCS) and glutamine. HeLa cells were used within 6 months of purchase from ATCC. HepG2, Huh7, HEK-VC and HEK-OCT1 cells were authenticated by short tandem repeat fingerprinting analysis (DSMZ, Braunschweig, Germany) and also regularly tested for mycoplasma using a PCR detection kit (Venor®GeM Classic, Minerva Biolabs GmbH, Berlin, Germany) as described.[1,2] Confocal laser scanning microscopy was performed on cells immune-labeled with an OCT1-specific

polyclonal rabbit antiserum (1:300).[1,3] Total RNA was isolated from liver cancer cell lines and transfected HEK cells as described[1] and *OCT1/SLC22A1* transcript levels were determined with a primer/probe assay as described.[1]

Statistical analysis

Data are presented as mean ± SD, unless stated otherwise. Group differences were evaluated using an unpaired t-test (2 groups) or one-way ANOVA (>2 groups) in GraphPad Prism 6, and the cut-off for statistical significance was set at $P<0.05$. Luminescence values of melting curves and isothermal inhibitor dose-response curves acquired with the InCell Pulse Target Engagement Assay were fitted to the sigmoidal dose response equation using Prism.

References

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