Mutual competition between imatinib and carnitine intake through OCTN2 in CML and muscular cells

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• Introduction:

The identified SNPs in regulatory regions of *SLC22A4* (*OCTN1*) and *SLC22A5 (OCTN2*) genes encoding influx transporters are associated with the response of CML patients to imatinib in the first line (Jaruskova et al. 2017). Moreover, the SNP rs460089 in the promotor of *SLC22A4,* was significantly associated with a probability of TFR in EURO-SKI patients after imatinib cessation (Machova et al. EHA 2019). *OCTN1* and *OCTN2* genes are probable evolutionary copies and the SNP rs460089 was identified to be in high linkage disequilibrium with seven other regulatory SNPs located in introns of both genes. Thus, the regulatory loci of the *OCTN1* may regulate expression of *OCTN2* and *vice versa*. This work focused on the imatinib intake efficacy by OCTN2.

• Methods:

Cells: KCL-22 (CML), HTB-153 (human rhabdomyosarcoma, ATCC). RT-PCR and the RT² Profiler™ PCR Array Human Drug Transporters. Intracellular concentration of imatinib and carnitines: quantitative LC-MS/MS MRM mode. Chromatographic separation- XBridge Amide column (150x2.1mm, 5µm; Waters, Milford (MA), USA) coupled to tandem MS QTRAP 4000 (Sciex, USA).

• Aim:

To test imatinib cell intake rate though OCTN1 and/or OCTN2.

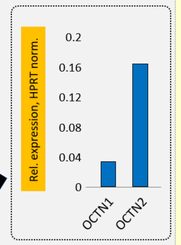
• Results:

1) OCTN2 (*SLC22A5*) is highly expressed in KCL-22 .

RT² profiler PCR array in KCL-22

ABC family		
median expression	1.42	N=16 genes
stdeva	2.85	
range (min-max)	0.10	9.86

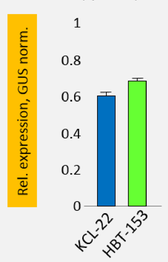
SLC family		
median expression	1.98	N=28 genes
stdeva	0.80	
range (min-max)	0.02	52.41



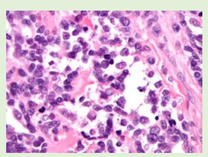
WB: OCTN2 (63.5 kDa)

*OCTN2 high expression is associated mainly with **muscle cells**, heart, brain cells etc.*

OCTN2 mRNA
(qPCR)



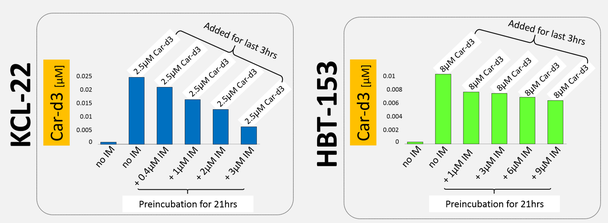
*Human rhabdomyosarcoma cells **HBT-153** used for comparison in experiments.*



2) Cell intake of carnitines is slower compared to imatinib (IM) intake.

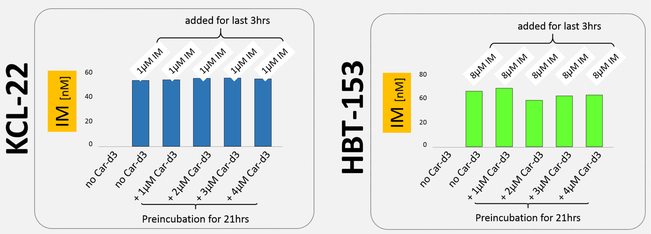
*To follow, whether imatinib is preferentially transported by OCTN2, the OCTN2 specific substrate **carnitine** was applied as a marker.*

3) Preincubation with IM reduces cell intake rate of carnitine.



Car-d3…isotopically labelled carnitine (to distinguish from carnitine dissolved in growth medium)

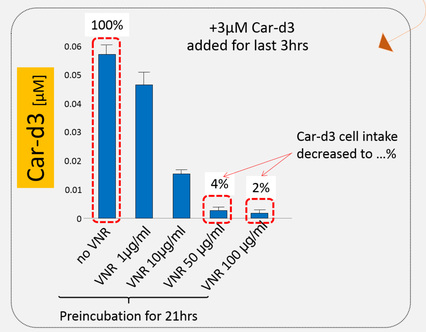
4) Preincubation with carnitine does not reduce cell intake rate of IM.



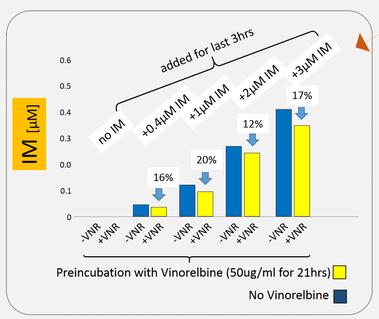
(*)…1 µM IM- sublethal dose for KCL-22 suspension cells
(**)… 8 µM IM- non-toxic dose for adherent HBT-153 (most suitable conc. for each individual cell availability)

5) OCTN2 inhibition by VINORELBINE (VNR):

- blocks carnitine cell absorption in KCL-22



- reduced IM cell intake in KCL-22



• Conclusions:

- **The OCTN2 specific carnitines intake was significantly reduced in the presence of imatinib in KCL-22 and HBT-153 cell lines**
- **High doses of carnitine in preincubation did not influence imatinib cell intake capacity**

- This observation is in line with the knowledge that imatinib is transported through other known imatinib transporters.
- The OCTN2 inhibitor vinorelbine inhibited imatinib intake down to 83%, supporting that **OCTN2** is a **member of imatinib transporters**.
- The observed non-equal competition between imatinib and carnitine intake can lead to the carnitine intracellular deficiency manifested by **a disruption of skeletal muscle mitochondrial density and can cause side effects like fatigue, muscle pain or cramp associated with rhabdomyolysis.** This hypothesis requires experiments focused on impact of carnitine deficiency caused by imatinib competitive intake on metabolism in muscle cells.

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