Floxuridine Prodrug Development: Increased Transporter Affinity and Enzymatic Activation

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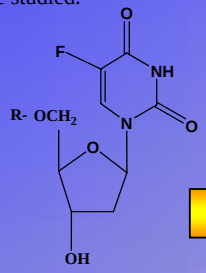
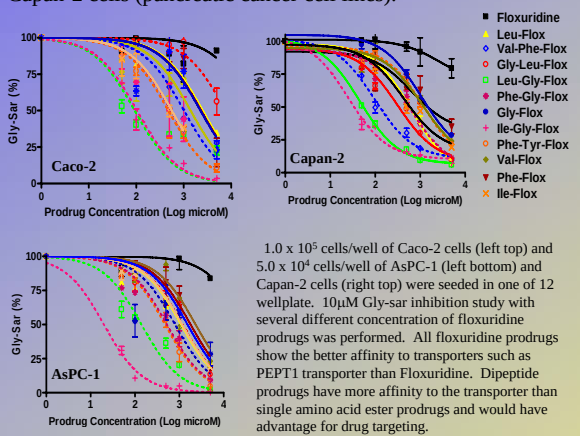
Purpose: Chemotherapy is a widely used treatment for various cancers. However, the non-selectivity of cancer drugs brings sever side-effects to cancer patients. Prodrug strategies are adopted for improving cancer treatments. In this presentation, the synthesis and the evaluation of prodrug approaches using floxuridine as a model drug in terms of the stability, the enhanced permeability via transporters, and the possibility of enzyme-targeted activation for improved cancer treatments were studied.



R= H- Floxuridine (5-FUrd)
R= Mono/Dipeptide Amino Acids

Fig 1. Floxuridine Prodrug

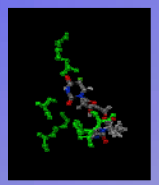
Suitability as a transporter substrate was assessed by their ability to inhibit [^{3}H]Gly-Sar uptake into Caco-2, AsPC-1 and Capan-2 cells (pancreatic cancer cell lines).



1.0 x 10^5 cells/well of Caco-2 cells (left top) and 5.0 x 10^4 cells/well of AsPC-1 (left bottom) and Capan-2 cells (right top) were seeded in one of 12 wellplate. 10μM Gly-sar inhibition study with several different concentration of floxuridine prodrugs was performed. All floxuridine prodrugs show the better affinity to transporters such as PEPT1 transporter than Floxuridine. Dipeptide prodrugs have more affinity to the transporter than single amino acid ester prodrugs and would have advantage for drug targeting.

Figure 2. Inhibitory Concentration 50% (IC50) of Prodrug for Gly-Sar Uptake

Table 1. The Stability of Glycosidic Bond Against Thymidine Phosphorylase



The active site of thymidine phosphorylase with Leu-Gly-Flox

Prodrug	Half Life (min)
Floxuridine	5.79 ± 2.99
5'-O-Phenylalanyl-Floxuridine	> 500
5'-O-Isoleucyl-Floxuridine	> 500
5'-O-Glycil-Floxuridine	249.61 ± 54.04
5'-O-Valine-Phenylalanyl-Floxuridine	> 500
5'-O-Leucin-Glycil-Floxuridine	137.98 ± 11.03
5'-O-Glycin-Leucil-Floxuridine	142.27 ± 10.37
5'-O-Phenylalane-Tyrosil-Floxuridine	> 500
5'-O-Isoleucin-Glycil-Floxuridine	223.34 ± 54.44

Floxuridine prodrug or floxuridine (Final conc.200μM) was incubated with human thymidine phosphorylase (TP) (0.024 units) at 37°C for over 2hours. 22μl of sample was taken up at the specific time points and mixed with 100μl of acetonitrile with 10% trifluoroacetic acid (TFA). The samples were spun at 10,000rpm at 4°C for 15min and the supernatant was filtered through glass microfiber filter, 1.0μm, Whatman®. Starting material and its metabolites were detected by HPLC. All prodrugs demonstrated the resistance against the enzyme, TP. Promoiety at 5′ position protects the glycosidic bond of floxuridine.

Table 2. Prodrug Stability in Cell Homogenates

	Leu-Flox	Gly-Flox	Phe-Flox	Val-Flox	Ile-Flox	D-Val-Flox
Caco-2	3.16 ± 0.23 min	24.07 ± 1.99 min	11.10 ± 9.87 min	9.39 ± 0.54 min	192.31 ± 31.84 min	412.78 ± 121.11 min
AsPC-1	1.95 ± 0.09 min	27.62 ± 5.80 min	11.81 ± 1.69 min	18.67 ± 6.70 min	197.99 ± 70.24 min	290.93 ± 48.94 min
Capan-2	4.65 ± 2.11 min	49.63 ± 5.60 min	2.99 ± 0.06 min	5.17 ± 2.39 min	209.74 ± 80.06 min	188.25 ± 12.10 min

	Leu-Gly-Flox	Gly-Leu-Flox	Phe-Gly-Flox	Phe-Tyr-Flox	Val-Phe-Flox	Ile-Gly-Flox
Caco-2	4.09 ± 0.11 min	25.39 ± 2.73 min	6.27 ± 0.64 min	86.23 ± 23.32 min	57.58 ± 9.29 min	20.52 ± 1.09 min
AsPC-1	3.63 ± 0.79 min	13.02 ± 1.36 min	10.22 ± 0.29 min	59.66 ± 1.39 min	51.57 ± 4.23 min	25.11 ± 5.79 min
Capan-2	3.93 ± 1.08 min	29.18 ± 0.74 min	4.26 ± 0.89 min	42.83 ± 0.03 min	56.18 ± 12.76 min	18.74 ± 1.43 min

Cells, which reach confluence on a 150mm plate, were rinsed with 0.9% NaCl twice and collected. Cells were suspended with 4ml of 100mM Potassium Phosphate butter, pH7.4, and were sonicated in ice-bath twice. The volume of the cell suspension was adjusted to 500μg/ml of protein amount. 2μl of 100mM prodrug was mixed with 998μl of the suspension (final drug conc. 200μM) and the mixture was incubated at 37°C for over 2hours. 30μl of sample was taken up at the specific time points and mixed with 100μl of acetonitrile with 10% trifluoroacetic acid (TFA). The samples were prepared on the same way as shown above (Table 1.) and were detected by HPLC. The results of prodrug stability in three different cell homogenates show that D-Val-Flox is the most stable and leucine incorporated prodrugs are the least stable.

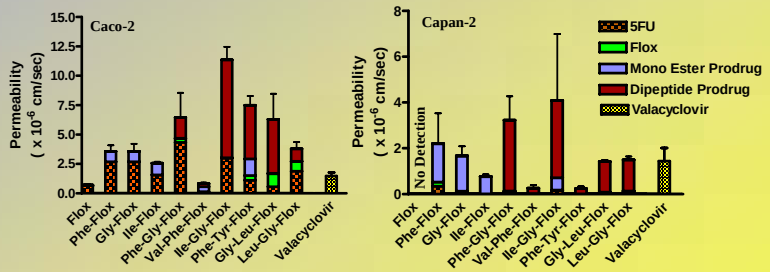


Figure 3. Caco-2 and Capan-2 Permeability of Floxuridine Prodrug and Floxuridine

1.2 x 10^5 cells/well of Caco-2 cells (left) and Capan-2 cells (right) were seeded and grown for 24days and 14days in a 6wellplate insert, respectively. 100μM test compound was applied on the apical side and the samples were taken out from the basolateral side for 2.5hours. The permeability was calculated with the appearance of test compounds and their metabolites. For the comparison purpose, valacyclovir was also studied.

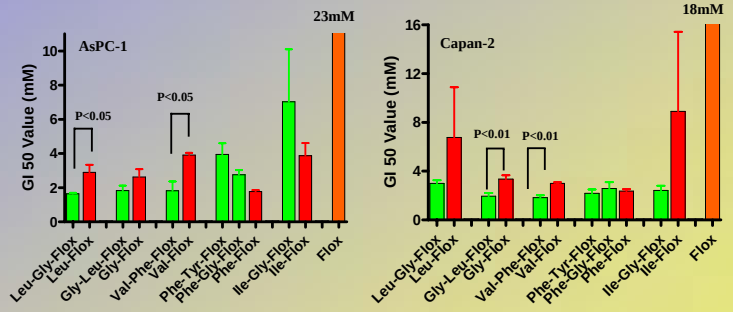


Fig 4. Cancer Cell Growth Inhibition 50% by Prodrugs and Parent Drug

1.2 x 10^5 cells/well of AsPC-1 cells (left) and Capan-2 cells (right) were seeded and grown for 24hours in a 96wellplate. 20μl of 0.25-4mM test compound was applied to each well and the plate was incubated at 37°C for 2hours and 4hours, respectively. Drug solution was removed and fresh media was added to each well. The cells were grown at 37°C for 24hours. The media was removed and 30μl of 1mg/ml XTT reagent with PMS in colorless media was applied to each well. The plate was incubated at 37°C for 1hour and the absorption was measured at 450nm with the reference at 805nm. The ability of tested compounds to inhibit cell growth was calculated and exhibited as the GI_{50} value.

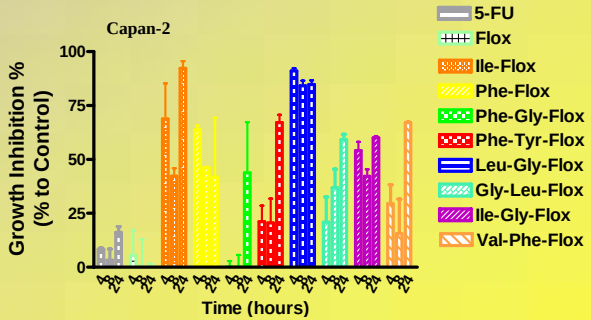


Figure 5. The Change of Growth Inhibition % with Different Interval

1.2 x 10^5 cells/well of Capan-2 cells were seeded and grown for 24hours in a 96wellplate. 20μl of 4mM test compound was applied to each well and the plate was incubated at 37°C for 4hours. Drug solution was removed and fresh media was added to each well. The cells were grown at 37°C for 4, 8, or 24hours. The growth inhibition was measure on the same way as shown above (Figure 4.). The ability of tested compounds to inhibit cell growth was calculated and exhibited as a growth inhibition %. The result exhibits that some of prodrugs need time to be activated and some are immediately activated and start inhibiting cell growth.

Conclusion: Floxuridine prodrugs demonstrate better affinity to transporters and permeability than floxuridine does. Increased drug delivery by prodrug strategy leads to better growth inhibition of pancreatic cancer cells. Upregurated enzyme activity in cancer cells quickly degrades floxuridine to less potent metabolites. Prodrug activation is varied due to their stability. Not only the permeability but also the improved drug stability of prodrugs may have great advantages for cancer treatment.

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