Structural Understanding of the Pgp1 Protein Using a Computationally Generated Binding and Motif Matrix for Improved Cancer Treatments.

Ellis Benjamin, Earl Benjamin, Louise Sowers, Christopher Saito, Tim Licknack, Shana Odlen, Peter Bruno, Rebecca Florre, Jackie Nghiem, Lauren Miller

The Department of Chemistry, The Richard Stockton College of New Jersey, 101 Vera King Farris Drive, Galloway, NJ 08205-9441

Abstract

P-glycoprotein 1 (Pgp), also called multidrug resistance protein 1 (MDR1), is one of the most widely dispersed and effective transporters found in cancer resistances. This research works sought to use computational methods to understand structure and functional relationship using the binding energies and structural similarity of the lowest binding molecules. A series of 479 molecules including alkaloids, Flavonoids, cyclic imides, lactams, lactones, NSAIDS, sulfanilamides, and known Pgp binders were bound to 3 Pgp crystal structures (35GU, 3G60, 3G61) . Computational results matched that of experimental result with a group of current pharmaceuticals which include Digoxin, Etoposide, Tacrolimus, and Paclitaxel maintaining the lowest energy (averaged over the three proteins). Similarity searches of the lowest binding molecules were conducted to determine important structural motifs. This research allows a better understanding of drug interaction towards the blockade of the function of Pgp.

Introduction

P-glycoprotein 1 (Pgp), also called multidrug resistance protein 1 (MDR1), is one of the most widely dispersed and effective ATP-dependent efflux transporters found in humans. The Pgp protein is found in multiple tissue types including those of intestinal epithelium, hepatocytes, renal proximal tubular cells, and capillary endothelial cells.[1-3] In addition, Pgp in conjugation with complex tight junctions comprise the physical and active transport system of the blood-brain and blood-testis barrier.[4]

The Pgp is used to remove toxic materials that cross cellular membranes including many lipophilic and inorganic drugs like Adriamycin, Vinca alkaloids, epipodophyllotoxins, actinomycin D, taxol, and cisplatin, to avoid cellular damage.[5,6] It is estimated that up to 50% of drug candidates may be substrates for Pgp. During times

of normal cellular function the Pgp works as a protection mechanism for the cell however during cancer treatment the Pgp counters the effectiveness of the chemotherapies given. The addition of chemotherapies also has the ability to increase Pgp production in cancer cells giving rise to chemotherapy resistances.[7] Specifically, the rapid activation of Pgp gene expression in human metastatic sarcoma has been found after in vivo exposure to doxorubicin.[8]

Researchers are currently working to find molecules which can work as sacrificial antagonist of Pgp to produce more effective chemotherapies. Molecules such as doxorubicin, vincristine, and etoposide are currently being used as Pgp sacrificial binders.[9,10] This research works sought to use computational methods to understand binding energies, active site interactions, and structural motifs similarity interactions of 479 molecules when bound to 3 Pgp crystal structures (1LWG, 1OY8, 1OY9) to gain a better insight into Pgp.

Experimental

iGEMDOCK by BioXGEM was used to determine protein –ligand docking interaction based on total binding energy, amino acid interaction energy, hydrogen bonding energy, and electrostatics. 8 categories of molecules were selected including Alkaloids (201 molecules), Flavonoids (37 molecules), Imides (73 molecules), Lactams (45 molecules), Lactones (36 molecules), NSAIDS (50 molecules), Sulfonamides (25 molecules), and known PGP binders (12 molecules). The binding energy totals for all three crystal structures (35GU, 3G60, 3G61) were averaged and ANOVA calculations were done using Excel with the statistical Analysis ToolPak Addin. Additionally, Graphpad Prism was also used to determine non-parametric ANOVA with a Dunn's post-hoc calculations. Protein clustering of amino acid binding energies was conducted using iGEMDOCK. Additional structural clustering by shape and electrostatics was conducted using vROCS © (Open Eye Scientific).

Results

Computational results matched that of experimental result with a group of current Pgp binders which include Digoxin, Etoposide, Tacrolimus, and Paclitaxel maintaining the lowest energy (averaged over the three proteins). This was confirmed using a one-way ANOVA when compared to the remaining 468 molecules. Additionally, a Dunn's post-hoc test found that known Pgp binders were significantly different (<0.001) than all all groups except Flavonids (P<0.01). 6 novel molecules were identified to bind comparable to known Pgp binders which included Alkaloids – 2 (-104.4668), Alkaloids – 586 (-100.4618), Flavinoids – 112 (-104.0059), Imides – 4 (-107.6884), Lactones – 1 (-101.8669), and NSAIDS – 20 (-103.7111). Active site interactions found that the region contain Ser 130 to Arg 144, Asp 160 to Val 164, and Ser 218 to Gly 222 active in the strong binding of these pharmaceuticals. This research allows a better understanding of drug interaction towards the blockade of the function of Pgp.

Conclusion

The computational calculation confirmed the experimental results with known Pgp binders statistically (P<0.001) being the best overall antagonist. Several amino acid regions were determine to be important to efficient binding which includes Ser 130 to Arg 144, Asp 160 to Val 164, and Ser 218 to Gly 222. Motif of known Pgp binders should provide the best estimation of sacrificial antagonist.

Overall Average and Standard Deviation					
Ligand	TotalEnergy	VDW	HBond	Elec	AverConPair
Average	-83.10362	-77.0488	-5.956476	0	25.84140286
Standard Deviation	24.2108256	24.38809	5.5305861	0	6.241218506
Averages					
Ligand	TotalEnergy	VDW	HBond	Elec	AverConPair
Alkaloids	-78.5163864	-70.5815	-7.888826	-0	23.41711987
Flavonoids	-82.1296157	-72.9949	-9.12705	-0	25.29726389
Imides	-79.4906434	-71.6404	-7.827904	-0	26.16178676
Lactams	-78.2154558	-69.2666	-8.913346	-0	25.52364917
Lactones	-56.1055296	-45.8388	-10.24001	-0	24.12709806
NSAIDS	-85.6107489	-79.4795	-6.025831	-0.1	25.02622815
Sulfonamides	-80.812248	-70.4709	-10.33373	-0	28.979408
PGP binders	-108.085314	-99.4954	-8.589974	0	17.76743667
Overall Standard Deviations					
Ligand	TotalEnergy	VDW	HBond	Elec	AverConPair
Alkaloids	48.7669379	48.86328	6.7836951	0.4	5.734380625
Flavonoids	17.8571395	17.33333	7.2259314	0.2	6.697908345
Imides	12.4133031	13.97518	6.2240068	0.3	5.1822988
Lactams	12.9640596	14.11066	6.0481711	0.3	6.479571636
Lactones	189.128651	186.7686	7.9821879	0.3	6.201334576
NSAIDS	10.3899718	13.59679	6.563287	0.6	5.340108989
Sulfonamides	10.3872608	11.83685	5.5986036	0.1	4.161624377
PGP binders	12.9814331	13.34128	6.8828687	0	4.907480877

ANOVA Excel

Groups	*Count*	*Sum*	*Average*	*Variance*
Alkaloids	201	-15546.2445	-77.3445	919.3108693
Flavonoids	37	-2956.666167	-79.9098964	358.787185
Imides	73	-5802.816967	-79.49064338	98.91562932
Lactams	45	-3128.618233	-69.52484963	734.4444324
Lactones	36	-2019.799067	-56.10552963	23499.42608
NSAIDS	50	-3878.130567	-77.56261133	489.2943849
Sulfonamides	25	-1898.821267	-75.95285067	77.78747855
PGP binders	12	-1256.333367	-104.6944472	134.4228851

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	27941.69502	7	3991.670717	1.731167295	0.099636098	2.029015298
Within Groups	1086016.882	471	2305.768326			
Total	1113958.577	478				

Graphpad Prism ANOVA and Dunns Post Hoc Test

Parameter	Value
Table Analyzed	
Data 1	
Kruskal-Wallis test	
P value	P<0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Do the medians vary signif. (P < 0.05)	Yes
Number of groups	8
Kruskal-Wallis statistic	39.85

Dunn's Multiple Comparison Test	Difference in rank sum	P value	Summary
Alkaloids vs Flavonoids	29.55	P > 0.05	ns
Alkaloids vs Imides	-9.208	P > 0.05	ns
Alkaloids vs Lactams	-45.29	P > 0.05	ns
Alkaloids vs Lactones	3.5	P > 0.05	ns
Alkaloids vs NSAIDS	16.24	P > 0.05	ns
Alkaloids vs Sulfonamides	-58.21	P > 0.05	ns
Alkaloids vs PGP binders	211.1	P < 0.001	***
Flavonoids vs Imides	-38.76	P > 0.05	ns
Flavonoids vs Lactams	-74.84	P > 0.05	ns
Flavonoids vs Lactones	-26.05	P > 0.05	ns
Flavonoids vs NSAIDS	-13.31	P > 0.05	ns
Flavonoids vs Sulfonamides	-87.76	P > 0.05	ns
Flavonoids vs PGP binders	181.5	P < 0.01	**
Imides vs Lactams	-36.08	P > 0.05	ns
Imides vs Lactones	12.71	P > 0.05	ns
Imides vs NSAIDS	25.45	P > 0.05	ns
Imides vs Sulfonamides	-49	P > 0.05	ns
Imides vs PGP binders	220.3	P < 0.001	***
Lactams vs Lactones	48.79	P > 0.05	ns
Lactams vs NSAIDS	61.53	P > 0.05	ns
Lactams vs Sulfonamides	-12.92	P > 0.05	ns
Lactams vs PGP binders	256.4	P < 0.001	***
Lactones vs NSAIDS	12.74	P > 0.05	ns
Lactones vs Sulfonamides	-61.71	P > 0.05	ns
Lactones vs PGP binders	207.6	P < 0.001	***
NSAIDS vs Sulfonamides	-74.45	P > 0.05	ns
NSAIDS vs PGP binders	194.8	P < 0.001	***
Sulfonamides vs PGP binders	269.3	P < 0.001	***

References

1. Sharom, F. J., The P-glycoprotein multidrug transporter. *Essays Biochem* 50, (1), 161-78.
2. K, C. L. L.; Rajaraman, G., Assessment of P-Glycoprotein Substrate and Inhibition Potential of Test Compounds in MDR1-Transfected MDCK Cells. *Curr Protoc Pharmacol* Chapter 7, Unit7 13.
3. Agarwal, S.; Mittapalli, R. K.; Zellmer, D. M.; Gallardo, J. L.; Donelson, R.; Seiler, C.; Decker, S. A.; Santacruz, K. S.; Pokorny, J. L.; Sarkaria, J. N.; Elmquist, W. F.; Ohlfest, J. R., Active Efflux of Dasatinib from the Brain Limits Efficacy against Murine Glioblastoma: Broad Implications for the Clinical Use of Molecularly Targeted Agents. *Mol Cancer Ther* 11, (10), 2183-92.
4. van Assema, D. M.; Lubberink, M.; Boellaard, R.; Schuit, R. C.; Windhorst, A. D.; Scheltens, P.; Lammertsma, A. A.; van Berckel, B. N., P-Glycoprotein Function at the Blood-Brain Barrier: Effects of Age and Gender. *Mol Imaging Biol*.
5. Breen, L.; Murphy, L.; Keenan, J.; Clynes, M., Development of taxane resistance in a panel of human lung cancer cell lines. *Toxicol In Vitro* **2008,** 22, (5), 1234-41.
6. Chou, T. C.; Guan, Y.; Soenen, D. R.; Danishefsky, S. J.; Boger, D. L., Potent reversal of multidrug resistance by ningalins and its use in drug combinations against human colon carcinoma xenograft in nude mice. *Cancer Chemother Pharmacol* **2005,** 56, (4), 379-90.
7. Chiou, J. F.; Liang, J. A.; Hsu, W. H.; Wang, J. J.; Ho, S. T.; Kao, A., Comparing the relationship of Taxol-based chemotherapy response with P-glycoprotein and lung resistance-related protein expression in non-small cell lung cancer. *Lung* **2003,** 181, (5), 267-73.
8. Abolhoda, A.; Wilson, A. E.; Ross, H.; Danenberg, P. V.; Burt, M.; Scotto, K. W., Rapid activation of MDR1 gene expression in human metastatic sarcoma after in vivo exposure to doxorubicin. *Clin Cancer Res* **1999,** 5, (11), 3352-6.
9. Yang, Z.; Wu, D.; Bui, T.; Ho, R. J., A novel human multidrug resistance gene MDR1 variant G571A (G191R) modulates cancer drug resistance and efflux transport. *J Pharmacol Exp Ther* **2008,** 327, (2), 474-81.
10. Lee, E.; Lim, S. J., The association of increased lung resistance protein expression with acquired etoposide resistance in human H460 lung cancer cell lines. *Arch Pharm Res* **2006,** 29, (11), 1018-23.