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P0964 | Lipophilic statins potentiate in vitro antitumoral activity of dihydrofolate reductase inhibitors: repositioning cholesterol-lowering drugs in Oncohematology

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Introduction

Dihydrofolate reductase (DHFR) inhibitors are antineoplastic and immunosuppressive drugs extensively used in the therapeutic of autoimmune diseases and cancer. However, when given in high doses, DHFR inhibitors (e.g., pemetrexed (PMTX)) can cause severe side effects and resistance. Statins, a widely used cholesterol-lowering drugs, display in vitro anticancer activity (e.g., antileukemic). However, it is unknown whether lipophilic statins (such as simvastatin (SV)) accumulate in tumor to induce cytotoxicity in vivo and what is the therapeutic window of statins that could safely be used in cancer patients. Several clinical trials have shown that high doses of statins need to be used to obtain the anticancer effect in vivo, which greatly limits its clinical use due to the derived toxicity. Combination of statins with conventional chemotherapy emerges as an efficient strategy that might increase therapeutic windows of statins in cancer [1]. Here we studied whether SV was able to potentiate the inhibitory effects of PMTX on cell viability in representative human leukemic cells.

Method

Human leukemic cells (HL60 (promyelocytic leukemia), MOLM13 (acute myeloid leukemia), Jurkat (immortalized T lymphocyte cell line), HEL (erythroleukemia) and K562 (chronic myelogenous leukemia)) were exposed to constant ratio combinations of SV with PMTX. Inhibition of cell viability was determined by the MTT assay. Dose-effect curves of single or combined drugs were plotted and analyzed by the median effect method as previously described [2], to obtain the combination index (CI) values.

Results

This preclinical study shows that SV and PMTX acted as potent antileukemic drugs on all human leukemic cell lines studied (Table 1). Moreover, SV effectively sensitized leukemic cells toward sub-toxic concentrations of PMTX resulting in synergistic inhibition of cell viability as demonstrated by reduced IC50 values when both drugs were combined compared to each drug alone, and CI calculations that indicated strong synergism for sub-toxic concentrations in all cell lines studied (Table 1).

Conclusions

Our results suggest that the efficacy of antifolate chemotherapy may be improved by combining sub-toxic concentrations with lipophilic statins. This combinatory strategy might increase therapeutic window and reduce drug resistance in oncohematolgy.

Table 1. Summary of Combination indexes (CIs) generated from the isobologram of increasing concentrations of pemetrexed (PMTX) and simvastatin (SV) (at least 6 doses) with a constant ratio combination design for HL60, MOLM13, Jurkat, HEL and K562 cell lines.

Combination	SV (μM)	CV_{SV} (%)	$IC50_{SV}$ (μM)	PMTX (μM)	CV_{PMTX} (%)	IC_{50PMTX} (μM)	CV_{Comb} (%)	$IC50_{CombPMTX}$ (μM)	CI (mean±SEM)
HL60									
1	0.04	100.0	1.6±0.08	0.0125	94.1	0.019±0.0001	74.3	0.016±0.001	0.12±0.01
2	0.08	100.0		0.025	41.5		39.0		0.10±0.02
3	0.15	100.0		0.05	38.0		21.6		0.11±0.03
4	0.3	94.5		0.1	37.9		24.9		0.21±0.02
5	0.6	77.5		0.2	37.1		22.0		0.37±0.02
6	2.2	51.5		0.4	36.0		23.0		0.97±0.05
7	2.40	26.9		0.8	35.6		22.8		1.27±0.16
8	4.80	21.0		1.6	35.6		15.9		1.98±0.17
MOLM13									
1	0.21	97.3	12.5±2.8	0.0002	96.7	0.013±0.004	97.0	$0.002±2.9e^{-5}$	2.02±1.28
2	0.43	96.5		0.0003	97.7		99.2		5.86±0.51
3	0.88	96.1		0.0006	93.7		93.1		0.85±0.28
4	1.75	92		0.0013	95.9		77.6		0.50±0.09
5	3.50	81.6		0.0025	96.7		46.5		0.27±0.05
6	7.0	63.8		0.005	86.2		30.7		0.32±0.11
7	14.0	43.9		0.01	80.1		14.9		0.35±0.19
8	28.0	26.7		0.02	42.1		8.3		0.47±0.32
9	56.0	15.2		0.04	26.4		5.3		0.84±0.70
Jurkat									
1	0.59	88.4	12.4±0.8	0.0005	95.9	0.025±0.01	84.1	0.004±0.0003	1.36±1.20
2	1.18	82.0		0.001	92.0		71.8		0.37±0.1
3	2.35	81.7		0.002	88.7		69.8		0.62±0.04
4	4.70	78.8		0.004	90.0		64.0		0.74±0.04
5	9.40	54.2		0.008	92.8		49.1		0.81±0.08
6	18.8	36.8		0.016	68.5		32.1		0.73±0.06
7	37.6	33.4		0.032	54.6		26.6		1.14±0.05
HEL									
1	0.112	95.7	19.1±4.8	0.0003	96.4	0.032±0.001	89.9	0.014±0.0002	0.32±0.19
2	0.234	97.6		0.0006	88.6		83.1		0.23±0.11
3	0.468	82.2		0.0013	95		78.4		0.34±0.18
4	0.93	79.5		0.0025	89		75.0		0.52±0.29
5	1.87	74.7		0.005	91.7		71.3		0.67±0.28
6	3.75	67.1		0.01	95.8		64.5		0.78±0.19
7	7.5	52.3		0.02	81.2		56.0		0.89±0.05
8	15	59.2		0.04	58.3		50.4		1.25±0.19
9	30	53.8		0.08	41.7		33.0		1.04±0.62
K562									
1	1.75	92.0	0.02 ±0.01	0.004	91.0	9.5±0.8	78.1	0.008±0.0002	0.68±0.37
2	3.5	68.2		0.008	68.4		56.4		0.53±0.08
3	7.0	48.7		0.016	58.5		37.3		0.63±0.04
4	14.0	35.1		0.032	51.6		28.0		0.98±0.21
5	28.0	30.4		0.064	50.3		18.9		1.51±0.29
6	56.0	28.0		0.128	50.9		20.0		3.17±0.11

IC50 values (μM) corresponding to the inhibition of cell viability determined by MTT assay after 48 h of treatment and combination indexes (CIs) (a.u.) are presented as mean ± SEM. CI less than 1.0 indicates synergistic effect, CI equal to 1.0 indicates additive effect and CI greater than 1.0 indicates antagonism. CV_{SV} indicates percentage of cell viability inhibition induced by simvastatin (SV) alone; CV_{PMTX} indicates percentage of cell viability inhibition induced by pemetrexed (PMTX) alone; CV_{Comb} indicates percentage of cell viability inhibition induced by PMTX in combination with SV. $CI50_{SV}$ indicates the half maximal inhibitory concentration for SV alone; $CI50_{PMTX}$ indicates the half maximal inhibitory concentration for PMTX alone; $CI50_{CombPMTX}$ indicates the half maximal inhibitory concentration for PMTX in combination with SV. Data are representative of at least four independent experiments with four replicates each.

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