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Chlorambucil synergizes with purine analogs in inducing *in vitro* cytotoxicity in B-cell chronic lymphocytic leukemia

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Combinations of different drug concentrations of CLB+FAMP and CLB+2-CDA were synergistic in, respectively, 42.9% and 34.8%. At leukemic cell survival ≤ 50%, 16.4% and 23.4% of all combinations were synergistic in the 2-CDA and FAMP groups, respectively. A significantly higher mean value of antagonistic interactions was observed in the 2-CDA group (p=0.037).

Fludarabine (FAMP), 2-chlorodeoxyadenosine (2-CDA) and chlorambucil (CLB) induce apoptosis in chronic lymphocytic leukemia (CLL) B-cells.[1,2] In this study we examined whether CLB improved *in vitro* CLL cell chemosensitivity to either FAMP or 2-CDA. The results indicate that CLB synergizes *in vitro* with both purine analogs.

Samples from 23 CLL patients were tested. Lymphocytes were separated as previously described.[3-5] CLB (Sigma, St Louis, Mo, USA), FAMP (Fludara, Schering AR, Germany) and 2-CDA (Leustatin, Ortho Biotech, USA) were employed at described concentrations.[3-5] MTT assay was performed as previously described.[3-5] The lethal dose (LD)[50] values, leukemic cell survival (LCS) and drug interactions were calculated by home made software.[3-5]

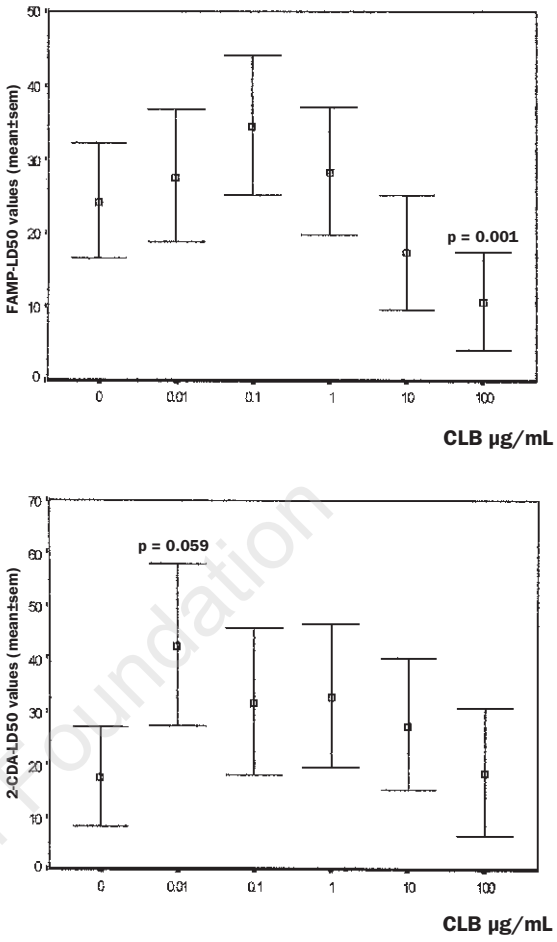

Figure 1. *In vitro* effect of several CLB concentrations on FAMP- (A) and 2-CDA- (B) LD[50] values at MTT assay. Statistical analysis was performed by Wilcoxon matched-pairs signed-ranks test. The difference of the mean values was significant between CLB= 0 µg/mL and CLB = 100 µg/mL (p=0.001) in the FAMP group. A border line significance was observed in the 2-CDA group between CLB = 0 µg/mL and CLB=0.01 µg/mL (p=0.0592).

FAMP-LD50 values were significantly lower with 100 µg/mL concentrations of CLB; conversely higher 2-CDA-LD50 values were observed with 0.01 µg/mL of CLB (Figure 1). The interactions between CLB and either 2-CDA or FAMP, tested in respectively 420 and 525 combinations, were synergistic in 146 (34.8% of the total) and 225 (42.9% of the total) (Table 1). Similar percentages of additive interactions (15.7% and 18.8%) were detected with both purine analogs, while a higher percentage of antagonistic interactions was observed among the 2-CDA group. At LCS ≤ 50%, 23%

Table 1. *In vitro* effect of drug interactions between CLB and FAMP in 21 CLL cases and between CLB and 2-CDA in 14. The mean values ± sem, the total number and the percentage of the total combinations in which each specific interaction was detected is indicated.

	Sy interactions mean values±sem (total, %)	**p*	*Ad interactions mean values±sem (total, %)*	**p*	*An interactions mean values±sem (total, %)*	**p*
Any LCS						
FAMP	10.7±1.4 (225, 42.9%)	ns	4.71±0.95 (99, 18.8%)	ns	9.57±2.6 (201, 38.3%)	0.037
2-CDA	10.4±2.1 (146, 34.8%)		4.71±1.1 (66, 15.7%)		14.85±2.6 (208, 49.5%)	
LCS ≤ 50%						
FAMP	5.8±1.0 (123, 23.4%)	ns	3.6±0.8 (77, 14.7%)	ns	3.2±0.7 (69, 13.1%)	0.059
2-CDA	4.9±1.4 (69, 16.4%)		3.4±0.9 (48, 11.4)		6.2±1.7 (87, 20.7%)	

*Sy interactions = Synergistic interactions; Ad interactions = Additive interactions; An interactions = Antagonist interactions; LCS= Leukemic cell survival. *Wilcoxon rank-sum test. For CLB+2-CDA interactions, 30 different drug combinations were tested using 5 CLB (100, 10, 1, 0.1, 0.01 μg/mL) and 6 2-CDA (125, 12.5, 1.25, 0.125, 0.0125, 0.00125 μg/mL) concentrations. For CLB+FAMP interactions, 25 different drug combinations were tested using 5 CLB (100, 10, 1, 0.1, 0.01 μg/mL) and FAMP (100, 10, 1, 0.1, 0.01 μg/mL) concentrations.*

and 16.4% of the total drug combinations in the FAMP and 2-CDA group, respectively, interacted synergistically. A statistically significant higher mean value of antagonistic interactions was observed in the 2-CDA group for any LCS (p=0.037), while a border line significance was documented for LCS ≤ 50% (p=0.059).

Synergy was observed between CLB and 2'-deoxycoformycin in CLL cells,[6] while an additive effect was described for the combination CLB-FAMP.[7] We found additivity and synergism in 61.7% and 50.5% for CLB+FAMP and CLB+2-CDA, respectively. On the other hand, there was a significantly higher mean value of antagonistic interactions in the 2-CDA group. Furthermore, in our *in vitro* tests, synergism and additivity was found in 60.7% of the drug interactions at CLB ≤ 1 μg/mL and 2-CDA ≤ 0.0125 μg/mL, which closely represent the purine analog plasma concentration achieved in a clinical study of the 2-CDA-CLB drug combination.[8,9]

In conclusion, these results adds support to the notion that combination of an alkylating agent with a purine analog may be a practicable treatment for CLL.

Key words

CLL, MTT assay, fludarabine, chlorambucil, 2-chlorodeoxyadenosine, cytotoxicity, drug synergism

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