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Valproic Acid Synergistically Enhances The Cytotoxicity of Clofarabine in Pediatric Acute Myeloid Leukemia Cells

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SUMMARY

Background—Acute myeloid leukemia (AML) remains a major therapeutic challenge in pediatric oncology even with intensified cytarabine (ara-C)-based chemotherapy. Therefore, new therapies are urgently needed to improve treatment outcome of this deadly disease. In this study, we evaluated antileukemic interactions between clofarabine (a second-generation purine nucleoside analog) and valproic acid (VPA, a FDA-approved agent for treating epilepsy in both children and adult and a histone deacetylase inhibitor), in pediatric AML.

Methodology—*In vitro* clofarabine and VPA cytotoxicities of the pediatric AML cell lines and diagnostic blasts were measured by using MTT assays. The effects of clofarabine and VPA on apoptosis and DNA double strand breaks (DSBs) were determined by flow cytometry analysis and Western blotting, respectively. Active form of Bax was measured by Western blotting post immunoprecipitation.

Results—We demonstrated synergistic antileukemic activities between clofarabine and VPA in both pediatric AML cell lines and diagnostic blasts sensitive to VPA. In contrast, antagonism between the two agents could be detected in AML cells resistant to VPA. Clofarabine and VPA cooperate in inducing DNA DSBs, accompanied by Bax activation and apoptosis in pediatric AML cells.

Conclusion—Our results document synergistic antileukemic activities of combined VPA and clofarabine in pediatric AML and suggest that this combination could be an alternative treatment option for the disease.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to disclose.

Keywords

pediatric acute myeloid leukemia; clofarabine; valproic acid; histone deacetylase inhibitor; synergistic antileukemic interaction

INTRODUCTION

Acute myeloid leukemia (AML) is a clinically and genetically heterogeneous disease accounting for 15–20% of childhood acute leukemias [1]. However, it is responsible for more than half of the leukemic deaths in this patient population. In contrast to the tremendous success in the treatment of acute lymphoblastic leukemia (ALL) over the last three decades, improvements in AML therapy have been modest, as 20–40% of patients do not respond to initial chemotherapy, and approximately 50% of responding patients will eventually relapse [2,3]. Therefore, new therapies for children with AML, especially those with primary refractory or relapsed disease, need to be developed. Among the newer agents that have been investigated in high-risk adult AML, clofarabine and histone deacetylase (HDAC) inhibitors (HDACIs) are particularly notable [4–7].

Clofarabine (2-chloro-9-[2′-deoxy-2′-fluoro-β-D-arabinofuranosyl]adenine; Cl-F-ara-A; CAFdA) is a rationally designed, second generation purine nucleoside analog [8–11]. Intravenous clofarabine showed significant efficacy in pediatric leukemias (specifically, ALL) [12]. Clofarabine was approved in December 2004 by the United States Food and Drug Administration (US FDA) for the treatment of pediatric patients with relapsed or refractory ALL after at least 2 prior regimens [8,11]. In adults, clofarabine has shown significant efficacy in hematologic malignancies including AML and myelodysplastic syndrome alone and in combinations [13–18].

HDACIs possess antitumor activities and have the potential to induce re-expression of genes abnormally suppressed in cancer cells, thus potentially inducing growth arrest, differentiation, and/or apoptotic cell death of transformed cells *in vitro* and *in vivo* [6,7,19–21]. Valproic acid (VPA) is a FDA-approved agent used for treating epilepsy in both children and adults, and was recently reported to be a powerful HDACI [22–24]. We previously demonstrated synergistic antileukemic activities of combined cytarabine and VPA in a panel of pediatric AML cell lines and diagnostic blast samples derived from children with *de novo* AML [25,26]. Cytarabine and VPA cooperatively induced DNA double-strand breaks (DSBs), as reflected in the induction of γH2AX (a biomarker of DNA DSBs), and apoptosis [25]. However, it is not yet established whether this represents a global mechanism that results in the synergistic cytotoxicity between HDACIs and DNA damaging agents in pediatric AML.

We hypothesized that VPA and clofarabine cooperate in inducing DNA damage and subsequent apoptosis in pediatric AML cells since clofarabine is also a DNA damaging agent. We demonstrate synergistic antileukemic activities of the two agents in VPA-sensitive pediatric AML cells. In contrast, antagonism between the two agents could be detected in VPA-resistant cells. In VPA-sensitive Kasumi-1 cells, clofarabine and VPA cooperate in inducing DNA DSBs and apoptosis, consistent with our previous findings with combined cytarabine and VPA in pediatric AML cells. These results suggest that this drug combination may be beneficial to AML cases which are sensitive to VPA.

MATERIALS AND METHODS

Clinical Samples

Diagnostic bone marrow samples (n=9) from children with *de novo* AML were obtained from the Children's Hospital of Michigan leukemia cell bank. Patient characteristics and blast percentages in each patient are summarized in Table I. Mononuclear cells were purified by standard Ficoll-Hypaque density centrifugation. Written consent was provided according to the Declaration of Helsinki. The Human Investigation Committee of the Wayne State University School of Medicine approved this study and the procedures involved.

Drugs

VPA was purchased from Sigma Chemical Company (St Louis, MO). Clofarabine was purchased from Tocris Bioscience (Ellisville, MO).

Cell Culture

The THP-1 (AML M5), Kasumi-1 (AML M2), and MV4-11 (AML M5) pediatric AML cell lines were purchased from the American Type Culture Collection (Manassas, VA). The CMS (AML M7 or AMkL) pediatric AML cell line was a gift from Dr. A Fuse from the National Institute of Infectious Diseases, Tokyo, Japan. The above cell lines were cultured in RPMI 1640 with 10–20% fetal bovine serum (FBS, Hyclone, Logan, UT) and 2 mM L-glutamine, plus 100 U/ml penicillin and 100 μg/ml streptomycin, in a 37°C humidified atmosphere containing 5% CO_2/95% air.

In Vitro Cytotoxicity Assays

In vitro clofarabine and VPA cytotoxicities of the pediatric AML cell lines and diagnostic blasts were measured by using MTT (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium-bromide, Sigma, St Louis, MO) assays, as previously described [25,26]. Briefly, the pediatric AML cell lines and diagnostic AML blasts were cultured in 100 μl of RPMI 1640/10–20% FBS in 96-well plates. For individual drug treatments, pediatric AML cells were treated with variable concentrations of clofarabine (0–1 μM, clinically relevant concentrations [27]) or VPA (0– 8 μM); while for combined treatments, the cells were treated with variable concentrations of clofarabine (0–1 μM) in combination with VPA (0–1 mM, clinically achievable concentrations [28]). After 72 hours, MTT was added to a final concentration of 1 mM. After 4.5 hours, formazan crystals were dissolved by the addition of 100 μl of 10% SDS in 10 mM HCl. Optical densities were measured with a visible microplate reader at 590 nm. IC_{50} values were calculated as drug concentrations necessary to inhibit 50% proliferation compared to untreated control cells. The extent and direction of clofarabine and VPA interactions were evaluated by standard isobologram analysis as described previously [25,29,30], and by using the CompuSyn software (ComboSyn, Inc., Paramus, NJ). Briefly, synergism, additivity, or antagonism was quantified by determining the combination index (CI), where CI<1, CI=1, and CI>1 indicate synergistic, additive, and antagonistic effects, respectively.

Growth Curve Analysis

Kasumi-1 cells were treated with clofarabine (5 nM) or VPA (0.5 mM) alone or combined for up to 96 hours. Viable cells were counted every 24 hours with trypan blue staining. Data was presented as means ± standard errors of triplicate determinations.

Assessment of Baseline and Drug-Induced Apoptosis

Kasumi-1 cells were treated with variable concentrations of VPA (0.25, 0.5, and 1.0 mM) and 5 nM clofarabine or variable concentrations of clofarabine (5, 10, and 20 nM) and 0.5

mM VPA alone, or in combination for 72 hours. The cells were harvested, vigorously pipetted and triplicate samples taken to determine baseline and drug-induced apoptosis using the Apoptosis Annexin-V fluorescein isothiocyanate (FITC)/propidium iodide (PI) Kit (Beckman Coulter; Brea, CA), as previously described [25,26,31]. Apoptotic events were recorded as a combination of Annexin-V+/PI− (early apoptotic) and Annexin-V+/PI+ (late apoptotic/dead) events and results were expressed as percent of Annexin-V+ cells.

Immunoprecipitation (IP) of Bax

To detect the active form of Bax, Kasumi-1 cells treated with clofarabine or VPA alone, or in combination, for 72 hours were lysed in Tris buffer (10 mM, pH 8.0) containing protease inhibitors (Roche, Indianapolis, IN). After determination of protein concentrations, cell lysates were diluted with the above Tris buffer to 1.33 μg/μl. After pre-clearing 400 μl of the sample with 20 μl Dynabeads® Protein G (Invitrogen, Carlsbad, CA) at 4°C for 1 hour, immunoprecipitation was performed by incubating 400 μl of the lysates with 4 μg of anti-Bax monoclonal antibody (clone 6A7, BD-Pharmingen, San Diego, CA; or B9, Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. After extensive washing with PBS containing protease inhibitors, Dynabeads-Ig-Antigen complex was heated at 70 °C for 10 min in 40 μl loading buffer, and 20 μl of the eluted proteins were analyzed by Western blotting.

Western Blot Analysis

Soluble protein extracts or immunoprecipitated proteins were subjected to SDS-polyacrylamide gel electrophoresis. Separated proteins were electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes (Thermo Fisher Inc., Rockford, IL) and immunoblotted with anti-Bax, -PARP, -cleaved-caspase 3, -γH2AX (Cell Signaling Technology, Danvers, MA), or -β-actin (Sigma, St Louis, MO) antibody, as described previously [32,33]. Immunoreactive proteins were visualized using the Odyssey Infrared Imaging System (Li-Cor, Lincoln, NE), as described by the manufacturer.

Statistical Analysis

Differences in clofarabine IC_{50}s (drug concentrations necessary to inhibit 50% proliferation compared to untreated control cells) between VPA-treated and -untreated AML cells and differences in cell apoptosis between clofarabine- and VPA-treated (individually or combined) and untreated cells were compared using the paired t-test. The relationship between apoptosis and levels of γH2AX was determined by the Pearson test. Statistical analyses were performed with GraphPad Prism 4.0.

RESULTS

VPA Synergistically enhanced clofarabine cytotoxicity in Kasumi-1 cells

To test our hypothesis that clofarabine and VPA synergize in their antileukemic acitivities against pediatric AML cells, we first evaluated the antileukemic activities of clofarabine in combination with VPA in Kasumi-1 cell line using MTT assays. When simultaneously administered with clofarabine, VPA at 0.15, 0.3 and 0.5 mM, significantly enhanced clofarabine sensitivities (reflected by the decreased IC_{50}s) by 1.4-, 2.1- and 4.2-fold, respectively (Figure 1A). The combined effects of clofarabine with VPA on cell proliferation were clearly synergistic, as determined by standard isobologram analysis (Figure 1C) and by calculating combination index (CI) values by using the CompuSyn software (Table II).

To determine the effects of treatment time on the antileukemic interactions between the two agents, Kasumi-1 cells were treated with 5 nM clofarabine or 0.5 mM VPA alone, or in

combination, for up to 96 hours, and cells were counted every 24 hours with trypan blue staining. As shown in Figure 1B, a minimum 48 hour co-treatment was needed to detect enhanced inhibition on cell proliferation. Interestingly, the synergism between clofarabine and VPA in Kasumi-1 cells was independent of the sequence of drug administration (Figure 1C).

Differential effects of VPA on clofarabine cytotoxicities in other pediatric AML cell lines and diagnostic blasts

To determine whether the synergistic antileukemic activities of VPA with clofarabine were unique to the Kasumi-1 subline, analogous cytotoxicity experiments were performed with MV4-11, THP-1, and CMS cell lines derived from children with different AML subtypes. Surprisingly, additive-to-synergistic (in MV4-11), additive (in CMS), or additive-to-antagonistic (in THP-1) results were obtained in these additional lines (Table II). At 0.5 mM VPA, simultaneous treatment with clofarabine resulted in 4.3-fold decrease in clofarabine IC_{50} in MV4-11 cells, compared to that from clofarabine alone. In contrast, VPA only had minimal effects on clofarabine IC_{50}s at this concentration in both THP-1 and CMS cells (Table II). Synergistic antileukemic activities (Table II) were detected in diagnostic AML blasts derived from 9 children with diverse subtypes of *de novo* AML (Table I), when VPA (0.15 to 1 mM) was administered simultaneously with clofarabine. Analogous to our previous study with cytarabine and VPA [25], diagnostic blasts from t(8;21) AML cases (n=3, patients 7–9) showed significantly increased sensitivities to clofarabine (range 18.4–52.5 fold) compared to those for non-t(8;21) AML cases (n=6, patients 1–6, range 1.3–15.2 fold) when combined with VPA at doses 0.5 mM or lower ($p<0.05$, Table II).

We then divided the AML cell lines and diagnostic blast samples into VPA-sensitive (IC_{50} ≤1.0 mM) and VPA-resistant (IC_{50} > 1.0 mM) groups by using the median VPA IC_{50} (1.0 mM) as the cut off. Interestingly, the VPA-sensitive AML cell lines and diagnostic blast samples showed 4.2- to 52.5-fold decreased clofarabine IC_{50}s when combined with VPA at doses 0.5 mM or lower. This is in great contrast to VPA-resistant cells which showed 1.1- to 15.2-fold decreased clofarabine IC_{50}s under the same experimental conditions ($p=0.014$, Figure 2). These results demonstrate that there are two groups of pediatric AMLs that show differential responses to the combination of clofarabine and VPA.

Cooperative induction of DNA DSBs and apoptosis by clofarabine and VPA in Kasumi-1 cells

Efforts were then undertaken to determine the molecular mechanisms that underlie the synergistic antileukemic interactions between the two agents. Our previous study demonstrated that VPA and cytarabine cooperatively caused DNA DSBs in Kasumi-1 cells [25]. It is conceivable that clofarabine and VPA may also cooperate in inducing DNA DSBs which subsequently trigger apoptosis since clofarabine is also a DNA-damaging agent. To test these possibilities, Kasumi-1 cells were treated with variable concentrations of clofarabine or VPA, alone or combined for 72 hours, and protein lysates were subjected to Western blotting to detect γH2AX. Consistent with our hypothesis, co-treatment with VPA and clofarabine resulted in cooperative induction of γH2AX, which was both clofarabine and VPA concentration-dependent (Figure 3). This was accompanied by cooperative induction of apoptosis determined by flow cytometry analysis and cleavage of caspase-3 and PARP, and activation of Bax in the cells (Figures 4A–E). Further, the levels of γH2AX significantly correlated with apoptosis ($r= 0.93$ and $p <0.0001$, Figure 4F). These results strongly suggest that VPA augments clofarabine-induced DNA DSBs which trigger apoptosis in Kasumi-1 cells.

DISCUSSION

In our previous study, we demonstrated global synergistic antileukemic activities of combined VPA/cytarabine in pediatric AMLs and suggested that VPA could be an attractive agent for combination therapies of this deadly disease [25]. In the present study, we investigated whether combining the novel nucleoside analog clofarabine with VPA could be an effective treatment option for children with AML.

Our initial results demonstrated synergistic antileukemic activities of combined VPA and clofarabine in a clinically-relevant pediatric AML cell line, Kasumi-1. Interestingly, this drug synergy was independent of the sequence of drug administration. We then expanded our study to include additional pediatric AML cell lines and diagnostic blasts from children with de novo AML. Consistent with our previous study [25], global synergistic results with clofarabine and VPA were obtained in the diagnostic blasts. In the additional cell lines (MV4-11, CMS, and THP-1), synergistic-to-additive and additive effects were observed in the MV4-11 and CMS cells, respectively, while additive-to-antagonistic effects were detected in the THP-1 cells (Table II). These results are different from our previous study which showed synergistic effects of combined VPA and cytarabine in THP-1 cells. The molecular mechanism underlying this difference is unknown. Nonetheless, it may reflect the different mechanisms of action between clofarabine and cytarabine in pediatric AML cells.

Of particular interest, responses of pediatric AML cells to the combination of clofarabine and VPA seemed to reflect the sensitivities of the cells to VPA (Figure 2). In addition, VPA-sensitive pediatric AML cells also showed significantly greater median fold change of cytarabine IC_{50}s than that of VPA-resistant cells when cytarabine was combined with VPA at 0.5 mM or lower concentrations (16.7- and 1.8-fold, respectively, p=0.008) [25]. Clinically, it would be difficult to estimate which pediatric AMLs are sensitive or resistant to the combination of VPA and clofarabine or cytarabine. Our results suggest, however, that sensitivities to VPA could be biomarkers for predicting responses of pediatric AML to this drug combination. In relation to this, we found that t(8;21) AML cases were uniformly sensitive to VPA, and so to the combination of VPA and clofarabine or cytarabine. This was not unexpected, given that several fusion proteins, such as AML1-ETO, recruit nuclear corepressor complexes, which contain HDACs, [34]. Thus, AML cases harboring these fusion genes might be preferentially susceptible to VPA and its combinations with clofarabine or cytarabine. Additional studies are warranted to determine the molecular basis underlying the differential responses of pediatric AML cells to VPA.

Our laboratory has previously showed that induction of apoptosis along with DNA DSBs was a major mechanism responsible for the anti-leukemic synergism between cytarabine and VPA [25]. Interestingly, this also seems the major molecular mechanism underlying the synergistic antileukemic interactions between clofarabine and VPA in pediatric AML cells. However, the molecular mechanisms by which VPA cooperates with clofarabine/cytarabine in inducing DNA DSBs and apoptosis remain unknown. Recent studies with solid tumor cell lines suggested that HDACIs can suppress the expression of DNA DSB repair genes, such as RAD51 and BRCA1 [7]. This could be the molecular basis underlying the synergy between VPA and clofarabine or cytarabine. Studies are underway investigating this possibility and results will be reported elsewhere.

Collectively, our results demonstrate synergistic antileukemic interactions between clofarabine and VPA in VPA-sensitive pediatric AML cells. Thus, children with VPA-sensitive AML may benefit from combination therapies involving VPA and clofarabine.

Acknowledgments

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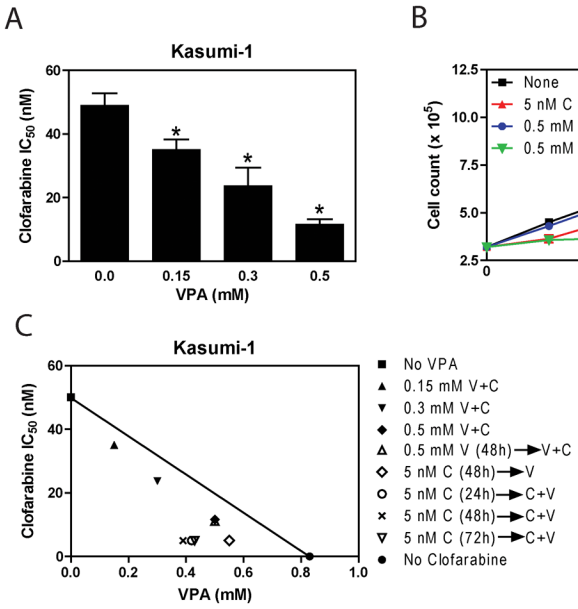

Figure 1. Synergistic antileukemic interactions between VPA and clofarabine in Kasumi-1 cells

Panel A: Kasumi-1 cells were cultured at 37 °C for 72 h in complete medium with dialyzed fetal bovine serum in 96-well plates at a density of 1.2×10^4 cells/well, with a range of concentrations of clofarabine and/or VPA, and viable cell numbers were determined using the MTT reagent and a visible microplate reader. The IC_{50} values were calculated as the concentrations of drug necessary to inhibit 50% proliferation compared to control cells cultured in the absence of drug. Clofarabine IC_{50}s of Kasumi-1 cells were determined in the absence or presence of VPA treated simultaneously. * indicates statistically significant difference ($p<0.05$). The data are presented as means ± standard errors from at least 3 independent experiments. **Panel B:** Kasumi-1 cells were treated with clofarabine (5 nM) or VPA (0.5 mM) alone or combined for up to 96 h. Viable cells were counted every 24 h with trypan blue staining. Data are presented as means ± standard errors of triplicate determinations. C, clofarabine; V, VPA. **Panel C:** Standard isobologram was used to analyze the antileukemic interactions between VPA and clofarabine in Kasumi-1 cells. The IC_{50} values of each drug are plotted on the axes; the solid line represents the additive effect, while the points represent the concentrations of each drug resulting in 50% inhibition of proliferation. Points falling below the line indicate synergism between drug combinations whereas those above the line indicate antagonism. → indicates pre-treatment with the first drug for the indicated times shown in parentheses. C, clofarabine; V, VPA.

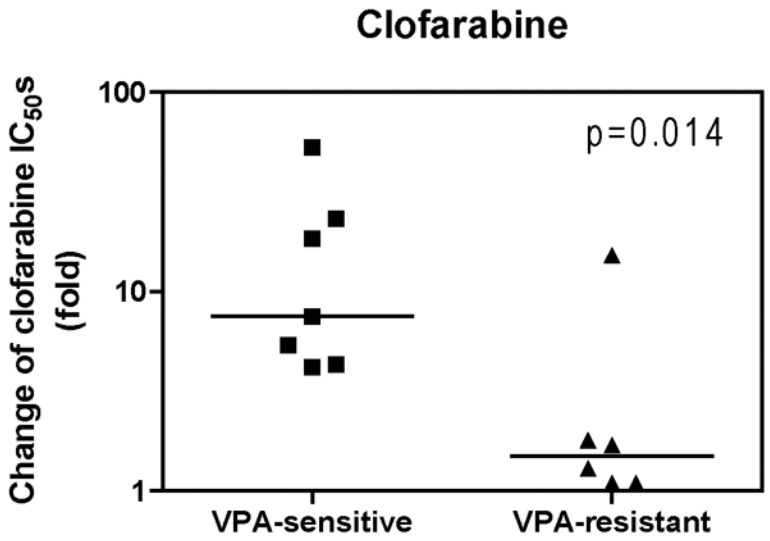

Figure 2. Differential responses of pediatric AML cells to the combination of clofarabine and VPA

The pediatric AML cell lines and diagnostic AML blast samples were divided into two groups based the median VPA IC_{50} (1.0 mM) measured by MTT assays. Cells with VPA IC_{50}s ≤ 1.0 mM were defined as VPA-sensitive, while cells with VPA IC_{50} > 1.0 mM were defined as VPA-resistant. Fold decrease of clofarabine IC_{50}s for pediatric AML cell lines and diagnostic AML blasts measured by MTT assays in the presence of 0.5 mM or lower VPA was compared with that from clofarabine alone. The horizontal lines indicate the median fold change in each group of AML cell lines and patient samples. The *p* value was determined by the nonparametric Mann-Whitney U test.

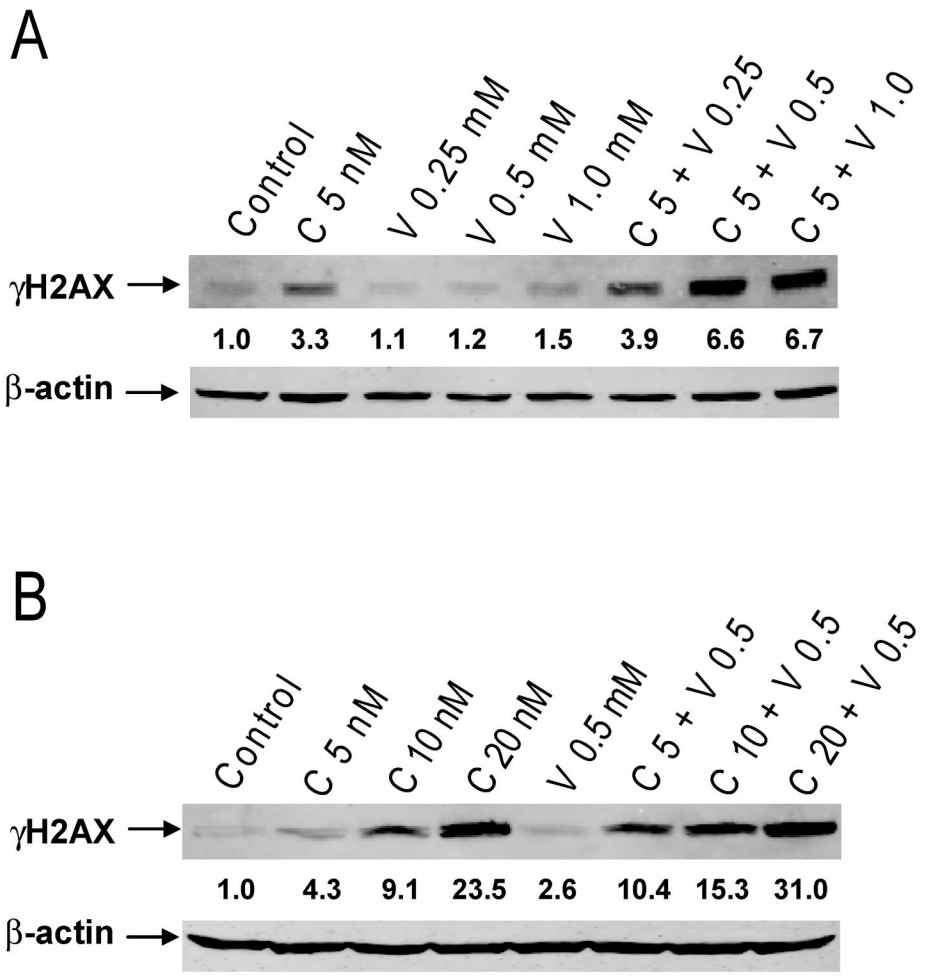

Figure 3. Cooperative induction of DNA DSBs by clofarabine and VPA in Kasumi-1 cells

Kasumi-1 cells were treated with variable concentrations of VPA and fixed concentration of clofarabine (**Panel A**) or variable concentrations of clofarabine and fixed concentration of VPA (**Panel B**) alone or in combination for 72 h. Whole cell lysates were extracted and subjected to Western blotting probed by anti-γH2AX or -actin antibody. C, clofarabine; V, VPA.

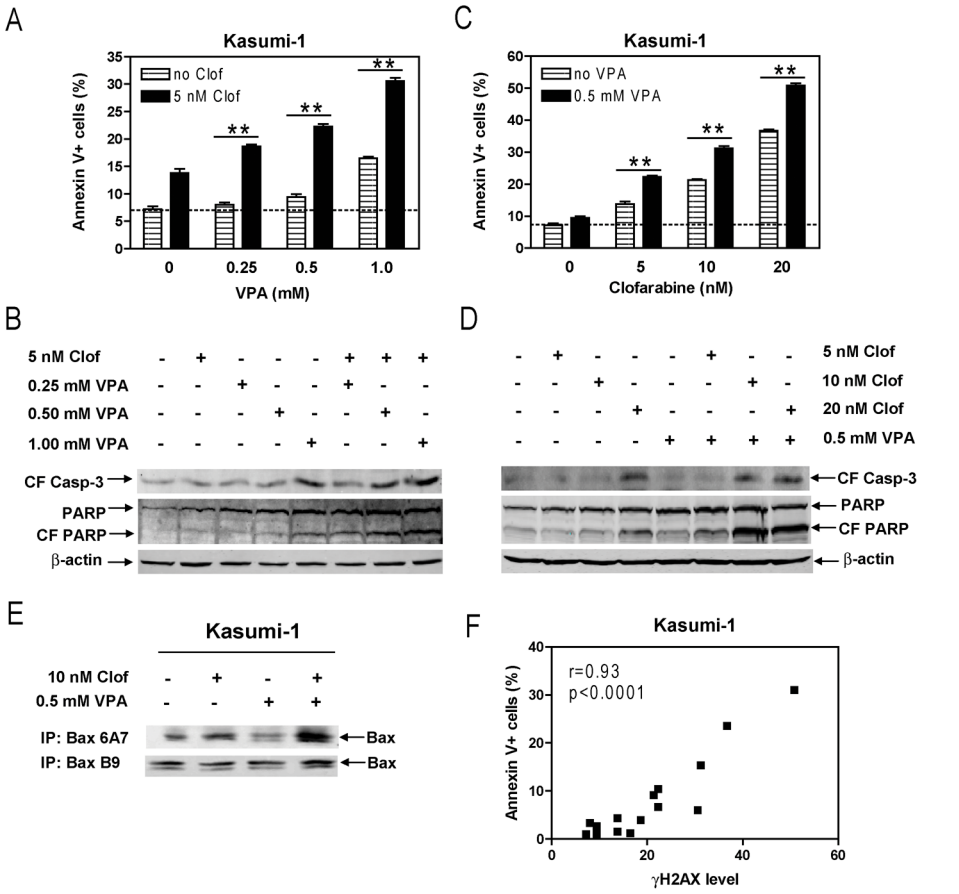

Figure 4. Cooperative induction of apoptosis by clofarabine and VPA in Kasumi-1 cells

Panels A–D: Kasumi-1 cells were treated with variable concentrations of VPA and fixed concentration of clofarabine (Panels A&B) or variable concentrations of clofarabine and fixed concentration of VPA (Panels C&D) alone or in combination for 72 h. Early and late apoptotic events in the cells post drug treatments were determined by annexin V/PI staining and flow cytometry analysis (Panels A&C). Whole cell lysates were extracted and subjected to Western blotting probed by anti-cleaved caspase-3 (CF casp-3), -PARP, or -actin antibody (Panels B&D). **Panel E:** Whole cell lysates from Kasumi-1 cells treated with clofarabine and VPA, alone or combined for 72 h, were subjected to immunoprecipitation with the Bax 6A7 (active Bax) or B9 (total Bax) antibody, as described in the Materials and Methods. The immunoprecipitated proteins were subjected to Western blotting probed by anti-Bax or -actin antibody. **Panel F:** The relationship between the levels for γH2AX and apoptosis in Kasumi-1 cells treated with clofarabine and VPA, alone or combined, were determined by the Pearson test. CF, cleaved form; Clof, clofarabine; ** indicates $p<0.005$.

Table I

Patient characteristics of pediatric AMLs used in the present study.

Patient ID	FAB	Age (year)	Sex	Cytogenetics	Blasts (%)
A30307	AML M6	10	Female	46, XX	29
A30308	AML M4/5	14	Male	46, XY, inv(16)	11
A30309	AML M4	0.8	Male	46, XY, inv(16)	30
A30310	AML M2	9	Male	46, XY	57
A30311	AML M4	14	Male	46, XY, t(3;5)	24
A30110	AML M5a	0.9	Male	46, XY, +9	73
A30207	AML M2	4.6	Male	46, XY, t(8;21)	82
A30224	AML M2	14.2	Female	46, XX, t(8;21)	48
A30230	AML M2	6.3	Female	46, XX, t(8;21)	39

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Table II

Effects of VPA on clofarabine cytotoxicities in pediatric AML cell lines and diagnostic AML blasts

Cell line/Patient	VPA IC_{50} (mM)	Clofarabine IC_{50} (nM)						*p* value
		0 mM VPA	0.15 mM VPA	0.3 mM VPA	0.5 mM VPA	0.75 mM VPA	1.0 mM VPA	
MV4-11	0.7±0.1	29.4±0.6	21.5±0.6 (0.9)	16.0±0.3 (1.0)	6.9±0.4 (1.0)	ND	ND	<0.008
Kasumi-1	0.9±0.1	49.0±3.7	35.0±3.1 (0.9)	23.6±5.6 (0.8)	11.6±1.5 (0.9)	ND	ND	<0.014
CMS	2.7±0.2	14.4±0.4	ND	ND	13.1±0.3 (1.0)	12.5±0.8 (1.0)	11.0±0.9 (1.0)	NS
THP-1	3.3±0.1	33.2±0.5	ND	ND	30.5±1.0 (1.0)	29.5±0.3 (1.1)	28.8±1.1 (1.1)	NS
A30307	1.1	18.2	ND	ND	1.2 (0.5)	0.8 (0.6)	ND	NA
A30308	4.9	337.8	ND	ND	259.0 (0.9)	191.1 (0.7)	148.3 (0.6)	NA
A30309	2.0	129.3	ND	ND	78.02 (0.8)	52.2 (0.9)	40.0 (0.8)	NA
A30310	1.7	38.9	ND	ND	22.2 (0.7)	12.8 (0.6)	13.2 (0.7)	NA
A30311	0.9	67.6	ND	ND	12.6 (0.8)	4.4 (0. 9)	ND	NA
A30110	1.0	55.9	ND	ND	7.5 (0.6)	3.2 (0.7)	ND	NA
A30207	0.7	36.8	ND	ND	2.0 (0.8)	ND	ND	NA
A30224	0.7	136.8	46.6 (0.5)	12.1 (0.5)	5.9 (0.7)	ND	ND	NA
A30230	0.2	189.1	3.6 (0.9)	ND	ND	ND	ND	NA

Note: For individual drug treatments, pediatric AML cells were treated with variable concentrations of clofarabine (0–1 μM) or VPA (0– 8 μM) for 72h; for combined treatments, the cells were treated with variable concentrations of clofarabine (0– 1μM) in combination with VPA (0–1 mM) for 72h. Viable cells were measured by using the MTT reagent and a visible microplate reader. Clofarabine and VPA IC_{50}s are presented as mean ± standard errors from at least three independent experiments for the pediatric AML cell lines, while those for the diagnostic AML blasts are presented as mean of duplicates from one experiment. Numbers in parentheses represent the combination index values. NA, not applicable; NS, not significant; ND, not determined.