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Research Article

Different Mitotic Arrest Targets of Chemoprevention Curcumin Analog 1.1 (CCA-1.1) and Pentagamavunone-1 (PGV-1) in Leukemic K-562 Cells

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Abstract

Background: Pentagamavunone-1 (PGV-1) is a promising cytotoxic chemotherapy agent in many cancer cells. However, due to its structure, PGV-1 is unstable and easily decomposed by light or high pH, causing decreased cytotoxic effect. Therefore, we developed a chemoprevention curcumin analog (CCA-1.1), a novel PGV-1 derivative which demonstrated improved solubility with similar antiproliferative activities toward breast and colon cancer cells. Our study intends to determine the antiproliferative effects based on the cellular and molecular mechanism of CCA-1.1 and PGV-1 in leukemic cells.

Methods: Using K-562 cells, CCA-1.1 and PGV-1 were tested for the cytotoxicity effect. Cell cycle analysis and ROS level were assessed by flow cytometry. Cells were stained with May–Grünwald–Giemsa and Hoechst to observe the mitotic phase arrest, while the X-Gal was selected to detect the senescence. The protein level of mitotic kinases (Aurora A, cyclinB1, and PLK1) was determined through Western blot.

Results: CCA-1.1 demonstrated an inhibitory effect on cell proliferation in K-562 cells following 96h treatment, with a GI_{50} value of 685 nM, akin to PGV-1 (GI_{50} score: 428 nM). Furthermore, this effect was found to be irreversible. It was shown that 1.2 μM CCA-1.1, similar to 0.8 μM PGV-1, induced cell cycle arrest specifically at mitosis after 24 h. CCA-1.1 induced cellular senescence and increased ROS production following 24 h incubation. A notable distinction between the two compounds lies in their respective effects on cell cycle progression. PGV-1 induced cell arrest at the prometaphase by 80% (p=0.0001), whereas CCA-1.1 was found to elicit around 20% of total cell arrest specifically at the metaphase (p=0.0035). Immunoblot experiments provided evidence that 24h treatment of CCA-1.1 tended to sustain the expression of p-cyclin B1, but PGV-1 led to an increase in the expression of p-cyclin B1 (p=0.0178) and p-PLK1 (p=0.0051).

Conclusion: The findings from our study provide evidence for the molecular mechanism on mitotic kinases of CCA-1.1 and PGV-1, resulting in the inhibition of the proliferation of leukemia cells during mitosis. Furthermore, CCA-1.1 induces mitotic catastrophe, leading to cellular death in K-562 cells.

Introduction

Chronic myeloid leukemia (CML), a frequent type of leukemia, is featured by the growth of granulocytic cell lines and the depleted capacity of cell differentiation. Due to the Philadelphia chromosome's translocation, CML is often associated with the continuous activation of the Bcr-Abl tyrosine kinase.[1] Despite many substantial breakthroughs in developing Bcr-Abl inhibitors (i.e. imatinib, dasatinib, nilotinib, etc.), remission remains short and thus leads to drug resistance; hence, new approaches must be urgently explored to overcome these hurdles.[2] As hematological cancers tend to have shorter tumor-doubling durations and an enhanced propensity to rapidly divide,[3] treatment with alternative classes of chemotherapy, such as antimitotic drugs, can be opted for in some cases. Therefore, the development of novel chemotherapeutic agents for CML is imperative.

In the last 15 years, synthesized substances based on

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curcumin, named pentagamavunone (PGV), have been explored for their anti-inflammatory activities.[4,5] Among those compounds, PGV-1 (Figure 1A) has been presented with more remarkable results than curcumin or another analog (PGV-0) for its anticancer activities against breast and colorectal cancer cells.[6–10] Similarly, PGV-1 exhibited a stronger antiproliferative effect than curcumin in CML K-562 cells.[11] Also, from the same study, PGV-1 was known to cause prometaphase arrest, bind with reactive oxygen species (ROS)-metabolizing enzymes, and lead to increasing the ROS level in cancer cells. Moreover, PGV-1 effectively prevented tumorigenesis on the xenograft model with an undetectable side effect.[11,12] Asides from its astonishing results demonstrated by PGV-1, as any other curcumin analog, it faces challenges when it comes to the solubility and stability in an aqueous solution for a long time. For that reason, the idea of modifying PGV-1 has been opted to overcome this problem without diminishing its antitumor effect.

Recent synthetic curcumin analog CCA-1.1 (Chemoprevention curcumin analog 1.1) is synthesized by mildly reducing the carbonyl site of the PGV-1 structure (Figure 1B), and it has been reported to be more soluble in aqueous buffer than PGV-1,[13] and at the same time also demonstrated antiproliferative activities against several types of cancer cells. This compound acts in various cancer cell processes, including the induction of mitotic arrest and cellular senescence, as well as the enhancement of reactive oxygen species (ROS) generation.[14-16] The bioinformatic research indicated that CCA-1.1 likely exerted its effects on mitotic kinases (Aurora A, polo-like kinase 1 (PLK1), cyclin dependent kinase 1 or CDK1) in triple-negative and HER2-positive breast cancer.[17,18] Furthermore, a subsequent investigation has shown that CCA-1.1 acted synergistically with PGV-1 to exhibit a cytostatic effect on K-562 cells.[19] Although PGV-1 has been shown to induce prometaphase arrest in CML K-562 cells,[11] no studies have explored the molecular activity of PGV-1 on mitotic kinases, nor have they investigated its effects on CCA-1.1. Since hematological tumors respond better to antimitotic drugs, presumably because they tend to have faster doubling times,[3] our study aims to investigate the molecular mechanisms of CCA-1.1 and PGV-1, focusing on cell cycle progression using CML K-562 cells.

Methods

Cell culture and compounds

A continuous K-562 cell line was cultivated in RPMI 1640 medium (Wako, Japan) and stored at 37°C with 5% CO_2. The chemotherapy imatinib (Glivec/STI571) was used as reference drug for CML. CCA-1.1 and PGV-1 were obtained from the Cancer Chemoprevention Research Center (CCRC), UGM, Indonesia. The chemical structure, synthesis, and purity of CCA-1.1 were described in the prior publication.[13] Throughout the experiments, the compounds were dissolved in dimethyl sulfoxide (DMSO) (Merck, Germany) and then added to a medium; the final concentration of DMSO was less than 0.5%.

Cytotoxicity and proliferative assay

Cell viability and cytotoxicity were examined by trypan blue exclusion.[20,21] The exponentially proliferating K-562 cells were treated with various concentrations of the tested compound. After 4 days following the treatment, the cell suspension was stained with 0.4% trypan blue (1:1 v/v) and directly counted using a hemocytometer for the non-stained cells. The viable cells were converted as a percentage of cell viability (of untreated cells) and calculated for a 50% growth inhibitory (GI_{50}) value.

A similar protocol was also applied for the proliferative assay. After treatment with CCA-1.1 (1.2 µM and 2.4 µM), the viable and dead cells were counted daily for total five days. For the washout experiment, the medium was replaced with a fresh one (without compound) after 2 days of exposure to CCA-1.1(1.2 µM) or PGV-1 (0.8 µM) and then observed for the next 2 days.

Flow cytometry-based assays

In brief, 3×10^5 cells/mL K-562 cells were incubated with the compound (1.2 µM CCA-1.1 or 0.8 µM PGV-1) at indicated times (24, 48, and 72 h), collected, and stained with propidium iodide solution (containing RNase A and Triton x-100) for 30 min before being filtered to prevent cell clumping. The cell cycle distribution was measured with FACS Calibur (BD Biosciences, USA) and analyzed using in-house Cell Quest software.

For ROS level determination, 1×10^5 cells were pretreated with 20 µM 2',7'-Dichlorofluorescin diacetate or DCFDA (Sigma Aldrich, USA) for 30 min and then with 0.8 µM PGV-1 or 1.2 µM CCA-1.1. The cells were filtered with a cell-strainer cap at indicated intervals before being subjected to flow cytometry.

Mitotic index determination

The treated (and untreated) cells were fixed into slides using cytospin. The slides were incubated with May–Grünwald (Merck, Germany) for 5 min and washed with phosphate buffer before being soaked in Giemsa solution (Merck, Germany) (1:20 v/v; diluted in phosphate buffer) for 20 min. Before observation under a phase-contrast microscope, the slides were rinsed in deionized water and dried in air.

For the mitotic spread assay, the cells pretreated with 1.2 µM CCA-1.1 (and 0.8 µM PGV-1) were incubated with 5.6% KCl for 6 min. After centrifugation, the cells were incubated with fixation solution (methanol: acetic acid with 3:1 v/v), dropped into slides (mitotic spread), and dried in air for 30 min. The slide was then incubated with Hoechst 33324 dye (Cell Signaling Technology, USA) (1:1000 v/v in PBS) for 1 h in a dark place and observed under a confocal microscope (Zeiss LSM710, Germany) with a violet 405 nm laser.

Senescence assay

After treatment for 24 h, the cells were placed on a slide using cytospin, rinsed with phosphate buffer saline (PBS), and fixed with 4% paraformaldehyde for 10 min. The slide was rinsed with PBS before being incubated with X-Gal (Wako, Japan) staining solution for 12 h in a 37°C incubator. The slide was observed under a phase-contrast microscope, and the blue-colored cells were indicated as positive senescent cells.

Immunoblot assay

In brief, 1×10^6 K-562 cells were treated with or without the tested compound (0.8 µM PGV-1 or 1.2 µM CCA-1.1) for 24 h. The cells were pelleted by centrifugation, washed twice with PBS, resuspended in 300 µL of lysis buffer (contained protease inhibitors), and centrifuged at 15,000 rpm and 4°C for 10 min to collect the supernatant. The same volume of cell lysate from each group was electrophoresed on 10% sodium dodecyl sulfate-polyacrylamide gels and then transferred to a PVDF (polyvinylidene difluoride) membrane (Millipore, Germany). The membrane was blocked in 5% bovine serum albumin (BSA) for 2 h. The primary antibodies used for this study were purchased from Cell Signaling Technology (SA) for phosphor-cyclinB1 (Ser133) (#4133), phosphor-PLK1 (Thr210) (#9062), phosphor-aurora A kinase (Thr288) (#3079), aurora A kinase (#12100), cyclinB1 (Santa Cruz Biotechnology, USA, sc-752), and PLK1 (Abcam, ab17056). β-actin antibody (Cell Signaling Technology, #3700) was used as a housekeeping protein. These primary antibodies were diluted in 5% BSA in tris buffer saline (TBS) solution (with 0.05% of NaN_3) (1:500 v/v), except for β-actin (1:1000 v/v). The membrane was probed with primary antibody overnight at 4°C with slow agitation. After being washed with PBS-Tween, the membrane was probed with an antimouse IgG secondary antibody (Cytiva, UK, NA931V) or protein-A HRP-linked antibody (Cytiva, UK, NA9120V) for 1 h at room temperature. After another PBST washing, the protein target was detected with a chemiluminescence kit (Cytiva, UK). Each band's intensity was semi-quantified and normalized by housekeeping protein (β-actin) through ImageJ software (NIH, USA).

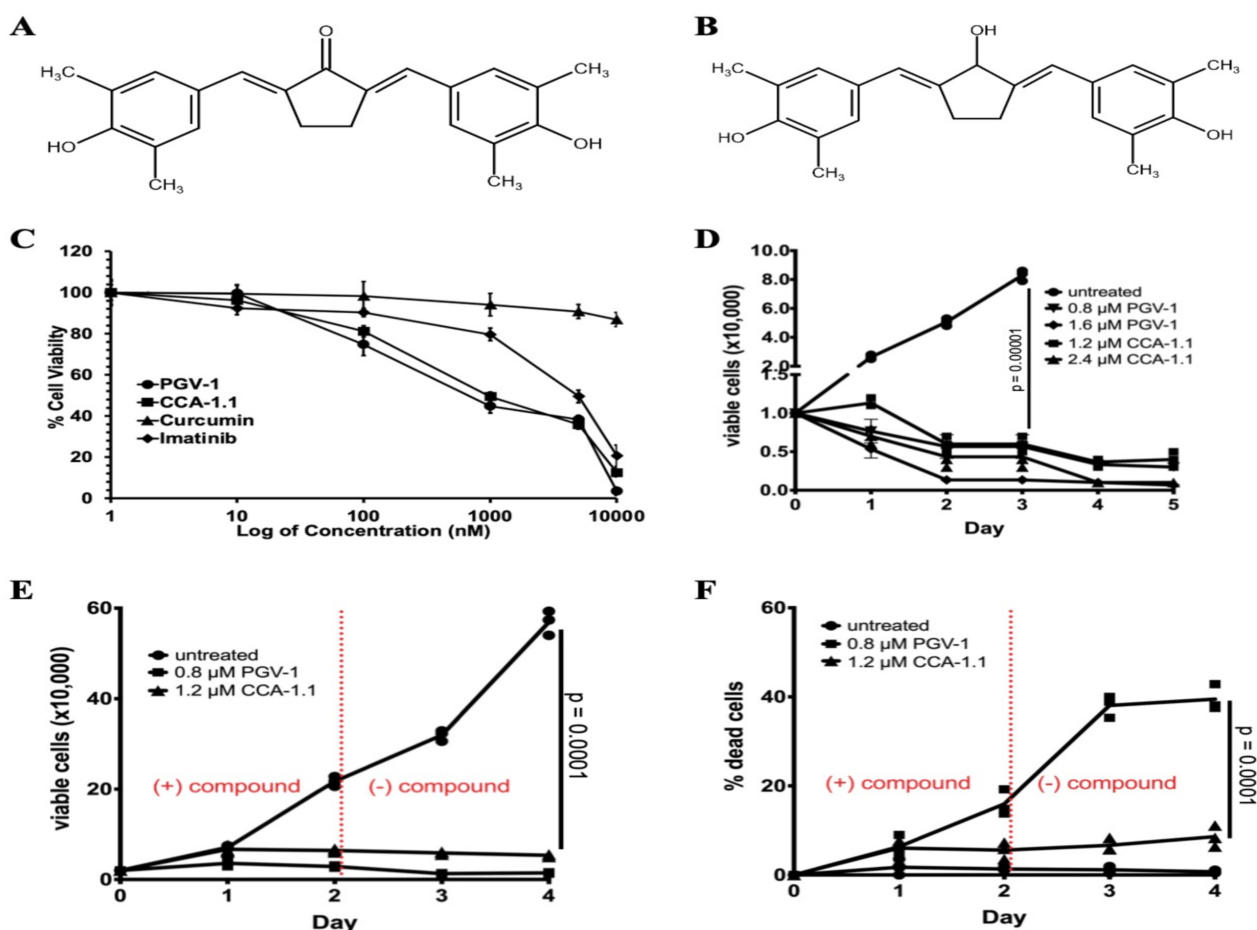

Figure 1. Inhibition of CML K-562 cell proliferation by CCA-1.1. and PGV-1. (A) Chemical structure of PGV-1; (B) Chemical structure of CCA-1.1; (C) The antiproliferative curve on K-562 cells after trypan blue assay with the GI_{50} score from PGV-1 and CCA-1.1; (D) The number of viable cells each day during treatment with sample throughout five days that enumerated by trypan blue exclusion method; (E) The number of viable; and (F) dead cells from washout experiment in K-562 cells. Each value represents the mean ± three replication. CCA-1.1: chemoprevention curcumin analog-1.1, PGV-1: pentagamavunone-1, GI_{50}: half growth inhibitory.

Statistical analysis

Data were presented as the average of three data ± standard deviation (SD) and examined by statistical analysis with ANOVA followed by Dunnett's multiple comparison test using GraphPad 9.0 (San Diego, CA, USA), with $p<0.05$ was considered statistically significant. All p-values were inserted in each figure.

Results

CCA-1.1 and PGV-1 inhibit K-562 cell growth

Cell viability assays showed that CCA-1.1 displayed a dose-dependent decrease in cell viability, with a GI_{50} value of 685 ± 143 nM, slightly higher than PGV-1, which had a GI_{50} value of 428 ± 70 nM. These cytotoxic effects were significantly stronger compared to the lead compound, curcumin, which did not affect K-562 cell growth at similar concentrations. Additionally, we compared the cytotoxic activity of these compounds with imatinib, a standard chemotherapy for CML, which had a GI50 value of 4.9 ± 0.7 μM (Figure 1C). This demonstrated that the curcumin analogs (PGV-1 and CCA-1.1) exhibit greater cytotoxic potency than imatinib. We then evaluate their antiproliferative effect on K-562 cells and counted the cells daily for total 5 days. Even at the lowest tested concentration (1.2 μM), CCA-1.1 drastically reduced viable cell growth ($p = 0.0001$), suggesting that CCA-1.1 impedes the growth of K-562 cells (Figure 1D). Based on this result, we chose 1.2 μM CCA-1.1 and 0.8 μM PGV-1 to be used in subsequent experiments.

To test whether removing the compound would induce the recurrence of cell proliferation, we replaced the PGV-1 or CCA-1.1-containing medium with a fresh medium after 2 days. Even with the removal of compounds, the inhibitory effect persisted, as indicated by the lack of an increase in viable cells (Figure 1E). Moreover, the percentage of dead cells increased despite the medium's absence of compound (Figure 1F). Therefore, CCA-1.1 demonstrates an irreversible antiproliferative effect on CML K-562 cells.

CCA-1.1 and PGV-1 similarly induce G2/M phase arrest of K-562 cells

After being exposed to 1.2 μM CCA-1.1, the distribution in the G2/M phase drastically elevated ($p = 0.0001$) from 23% to 40% during the first 24 h (Figure 2A). Compared with 0.8 μM PGV-1 treatment, where most cells (including G0/G1 peak) diminished according to the flow cytogram profile and most cells were drastically ($p = 0.0001$) induced G2/M arrest by 75%, the CCA-1.1 treatment induced a different cell profile. In CCA-1.1-treated cells, G0/G1 cell population was present but decreased in percentage from 52% to 33% in the first 24 h. We then checked whether CCA-1.1 or PGV-1-induced G2/M arrest remained stable in longer exposure (48 and 72 h). After prolonged incubation, CCA-1.1 drastically ($p = 0.0001$) induced the accumulation of sub-G1 population (21% versus 6% from untreated) at 72 h, indicating cellular death due

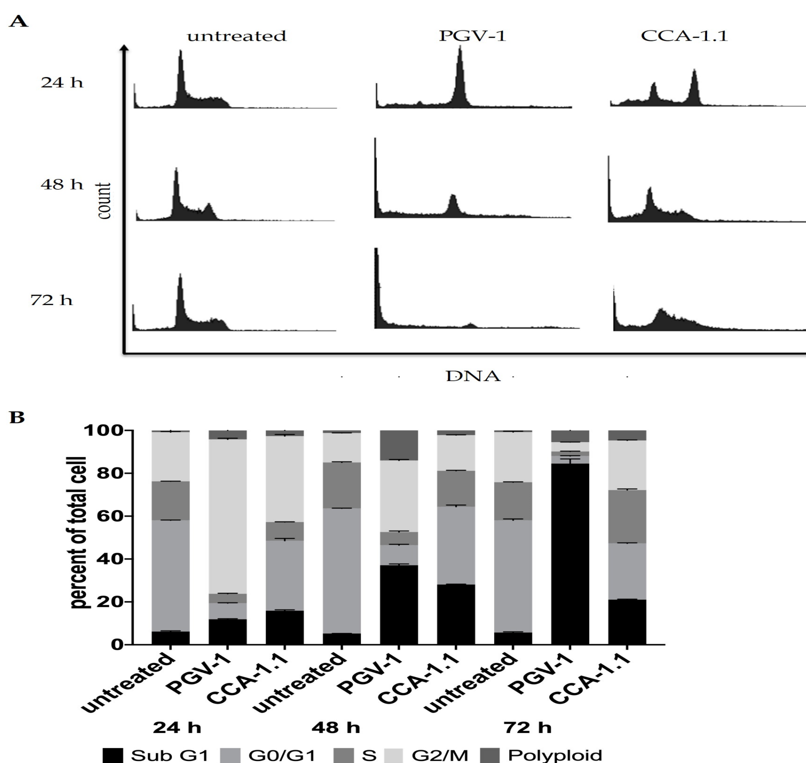

Figure 2. Increase of G2/M phase cells and subG1 cell population distribution by CCA-1.1 and PGV-1. (A) The flow cytogram of untreated, 0.8 μM PGV-1, and 1.2 μM CCA-1.1-treated cells; and (B) The proportion of cell cycle phase based on the flow cytometry analysis. Values are displayed as mean ± SD from three data. CCA-1.1: chemoprevention curcumin analog-1.1, PGV-1: pentagamavunone-1.

to chromosomal fragmentation (Figure 2B). In PGV-1 treatment, extended exposure caused the accumulation by 80% at 72 h observation, indicating that the prolonged mitotic arrest resulted in cell death. This finding suggested that CCA-1.1 blocks the cell cycle during the G2/M phase.

CCA-1.1 and PGV-1 induce cellular senescence and ROS production in K-562 cells

Considering that 24 h treatment of CCA.1.1 affected the cell cycle to inhibit cell proliferation in G2/M phase, we further explored whether CCA-1.1 induces the senescence of K-562 cells. After 24 h incubation with CCA-1.1 (1.2 µM), the number of senescent cells increased due to lysosomal β-galactosidase activity observed in the CCA-1.1-treated cells (Figure 3A). Further quantification demonstrated that CCA-1.1 dramatically induced senescence almost threefold compared with the control cells, similarly, demonstrated with PGV-1 (0.8 µM) treatment (Figure 3B).

The presence of senescent cancer cells is often associated with chemotherapy, which also triggers the accumulation of cellular ROS. To elucidate whether CCA-1.1 treatment enhances ROS generation, we incubated K-562 cells with CCA-1.1 and performed flow cytometry at several indicated times. The number of fluorescent DCF molecules, an oxidation product from cellular ROS, increased upon treatment with CCA-1.1. Cellular ROS level was low in the first 2 h of incubation but rapidly increased by twofold against the untreated group starting at 4 h. The high ROS level induced by CCA-1.1 was maintained until 24 h (except during 18 h) of post-treatment, while drastically ($p = 0.0001$) decreased in PGV-1-treated cells (Figure 3C). In short, the action of CCA-1.1 on cellular senescence is mediated in part by the production of intracellular ROS.

CCA-1.1 and PGV-1 distinctly target different phases in mitosis

Considering the cell cycle profile with the arrested accumulation of G2/M cells after CCA-1.1 treatment in 24 h, we stained the cells using May–Grünwald–Giemsa (MGG) solution and Hoechst fluorescent dye. The incubation of CCA-1.1 (and PGV-1) for 24 hours induced mitosis rather than G2 arrest. Moreover, most mitotic cells entered the metaphase on CCA-1.1-treated cells, while PGV-1 induced mitotic arrest at prometaphase (Figure 4A). After quantification, 20% of cells were arrested in mitosis due to CCA-1.1 treatment, followed by 80% of cells halted in prometaphase after PGV-1 treatment (Figure 4B). Consistent with this result, Hoechst staining also presented

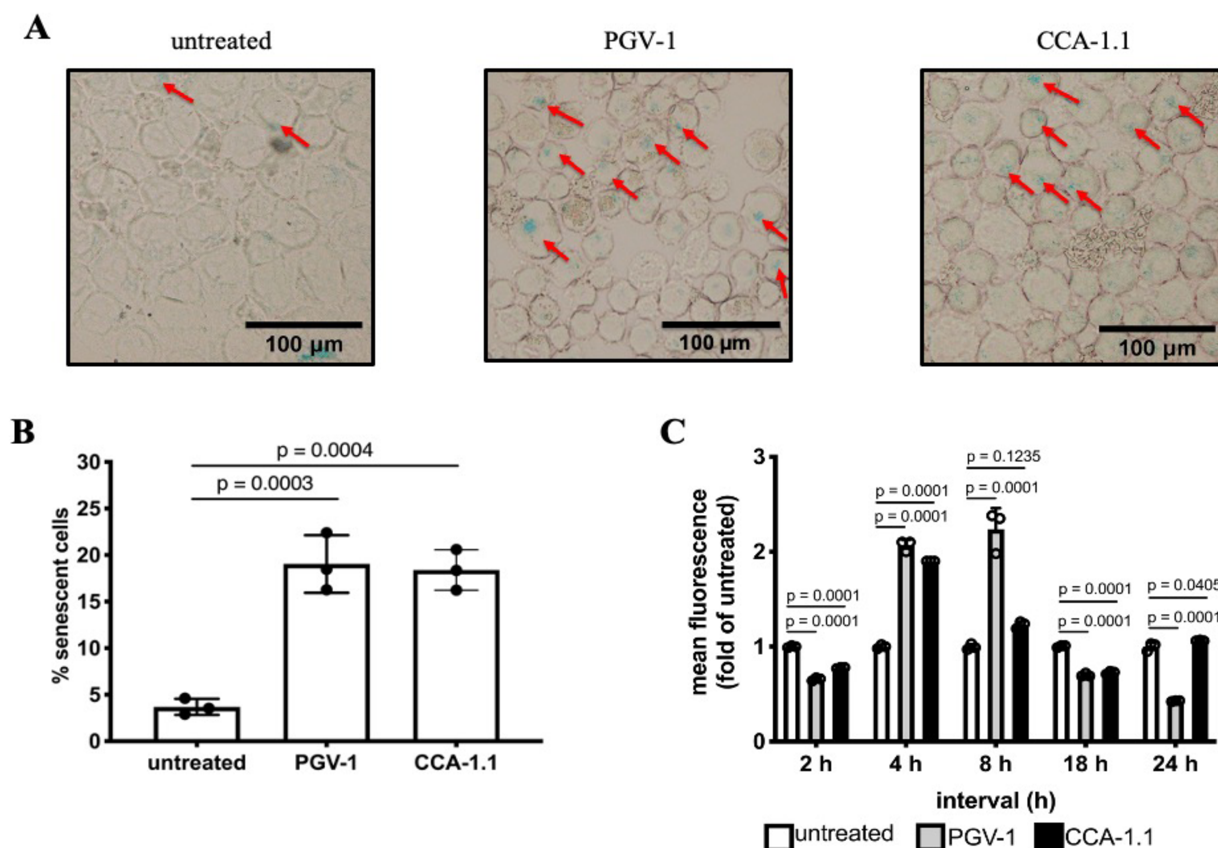

Figure 3. Increase of lysosomal β-galactosidase activity and ROS level in K-562 cells by CCA-1.1 and PGV-1. (A) Cells morphology after X-Gal staining under microscope (total magnification 400×). (B) Quantification of blue-colored cells as senescent cells. (C) The fluorescence from the oxidation of DCFDA, which indicates ROS level, was normalized against the untreated group. Values are displayed as mean ± SD from three data. CCA-1.1: chemoprevention curcumin analog-1.1, PGV-1: pentagamavunone-1, ROS: reactive oxygen species.

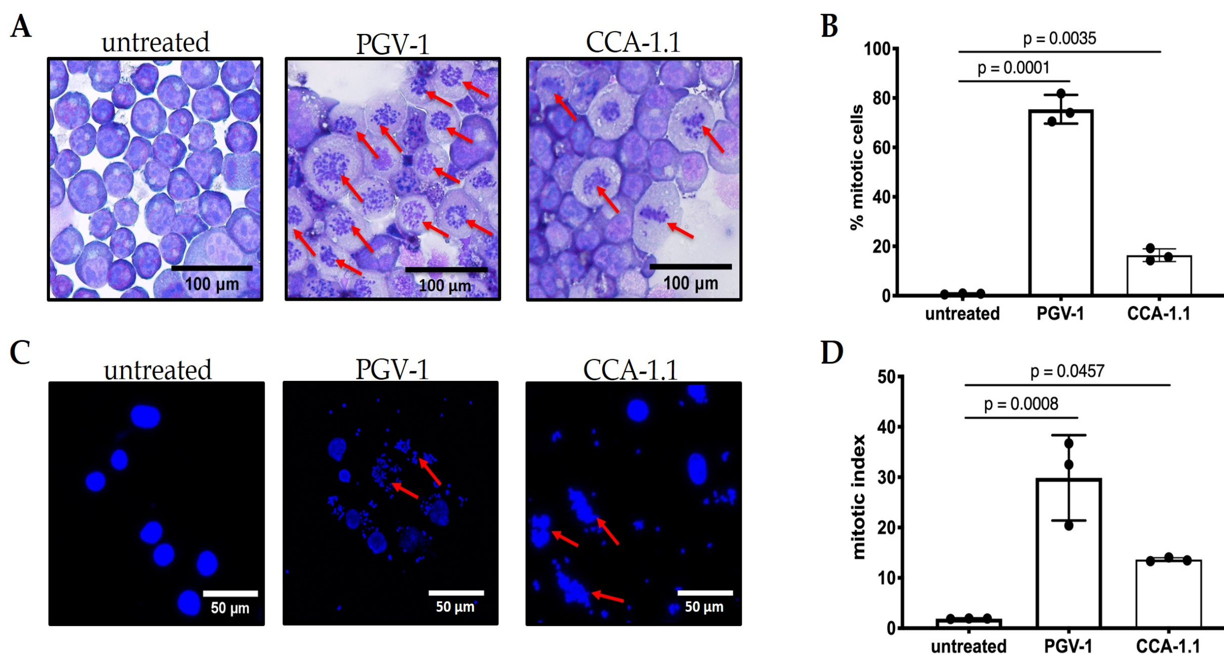

Figure 4. Induction of mitosis arrest using CCA-1.1 and PGV-1 in a different phase. (A) Cells after May–Grünwald-Giemsa (MGG) staining under a phase-contrast microscope (total magnification 400×); (B) Percentage of mitotic cells from MGG-stained cells; (C) PGV-1 or CCA-1.1-treated cells were also analyzed through mitotic spread assay (total magnification 200×); (D) Mitotic cells were quantified using the mitotic parameter index. Values are displayed as mean ± SD of three data. CCA-1.1: chemoprevention curcumin analog-1.1, PGV-1: pentagamavunone-1.

a similar effect as indicated by the metaphase arrest after incubation with CCA-1.1 and PGV-1 (with prometaphase arrest) for 24 h (Figure 4C) that led to a significant (p = 0.0457) value of the mitotic index (Figure 4D). In summary, these curcumin analogs halt cell progression in different phases during mitosis.

CCA-1.1 and PGV-1 differentially regulate the cell cycle-associated proteins in K-562 cells

Given that 24 h treatment of CCA-1.1 that also acted in inducing mitotic arrest based on the cell cycle profile and chromosomal staining, we identified the specific mechanisms mediated by these substances to regulate the mitosis of CML K-562 cells. Since mitotic progression is primarily activated through phosphorylation on serine or threonine residue,[20] we opted to examine whether mitotic kinase activation is influenced by PGV-1 and CCA-1.1 activity. First, we checked the protein expression of treated cells using Western blot (Figure 5A). Although p-Aurora A expression level was not altered after treatment with both compounds (Figure 5B), only PGV-1 increased the phosphorylation of cyclin B1 (Figure 5C). Moreover, PGV-1 significantly (p = 0.0051) induced phosphorylation of PLK1 in K-562 cells (Figure 5D). These results demonstrated that although they shared the ability to cause mitotic arrest, PGV-1 and CCA-1.1 functioned in distinct ways on the molecular level in K-562 cells.

Discussion

Curcumin and many of its derivatives or analogs can suppress cell growth in CML-derived K-562 cells.[11,21-24] They also impact tumor progression by preventing cell cycle progression and inducing cellular death. Regardless of their remarkable results in cancer cells, these analogs encounter hurdles mainly because of their low gastrointestinal bioavailability, contributing to their less efficacy.[24] Hence, several attempts have been made to improve the stability of curcumin by generating its analogs. Previously, we synthesized a curcumin analog, PGV-1, and observed its promising potency as an anticancer in various cancer cells, including leukemic cells, K-562. However, due to its structure, PGV-1 is unstable and readily decomposed by light or high pH. Therefore, we developed CCA-1.1 to improve the stability of PGV-1.[13] The potency of CCA-1.1 as an anticancer agent against breast and colorectal cancer cells has been observed, demonstrating favorable activity.[14,25-27] Regarding its selectivity, PGV-1 was relatively less cytotoxic when tested in fibroblast[11,28] a phytopolyphenol found in turmeric (Curcuma longa and similar phenomenon was also found following CCA-1.1 treatment[27] given by a selectivity index greater than 3, highlighting its good selectivity toward cancer cells. Larasati *et al.*[22] previously reported that curcumin had a stronger irreversible effect on K-562 cell proliferation than imatinib, indicating that curcumin outperformed imatinib. This time, we discovered that curcumin analogues PGV-1 and CCA-1.1 are more cytotoxic in CML K-562 cells than curcumin.

This study compared molecular evidence of CCA-1.1 efficacy against K-562 cells with PGV-1. Our study revealed that CCA-1.1 induces cell cycle arrest in K-562 cells during mitosis, which is quite different compared

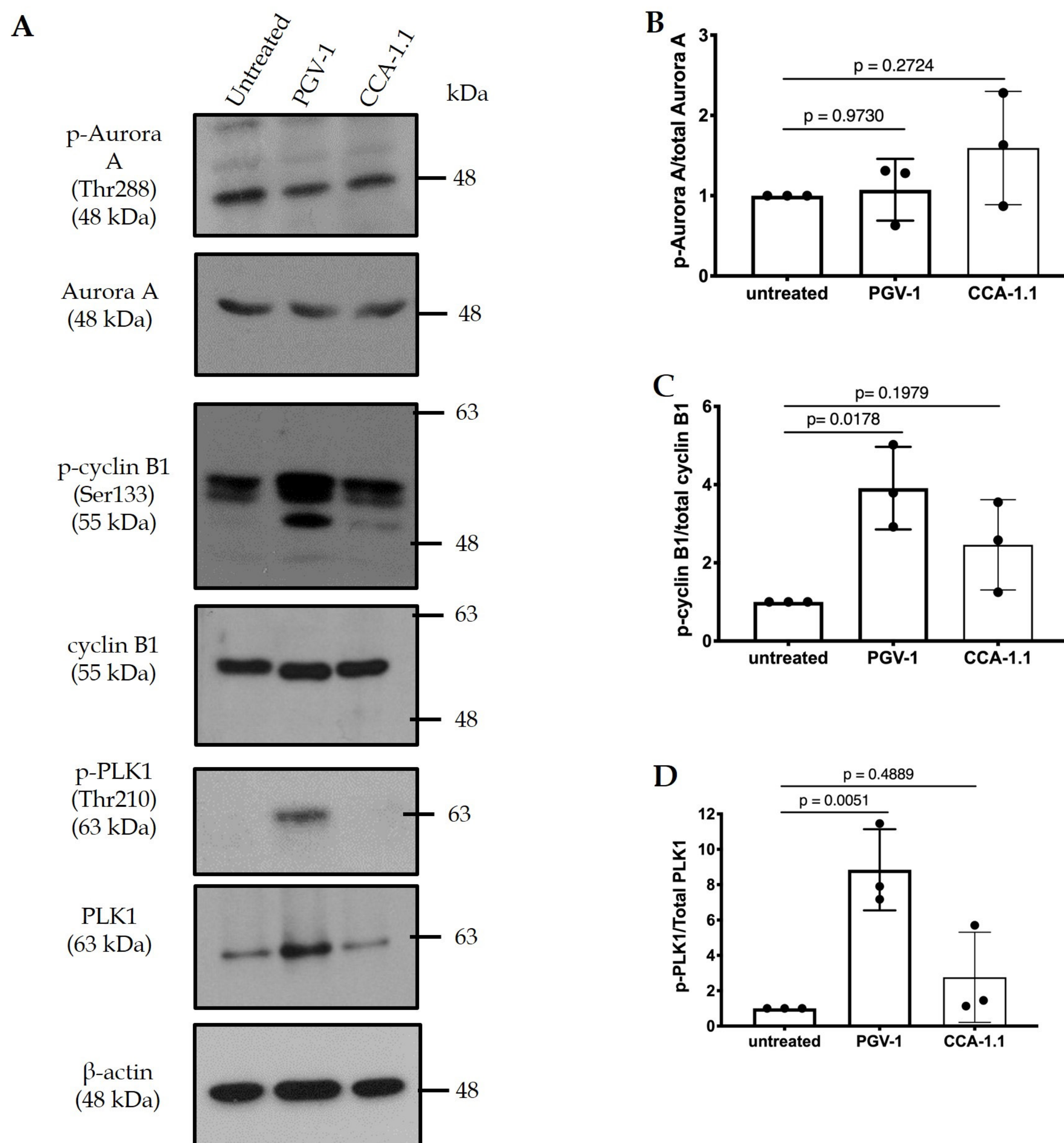

Figure 5. Effect of PGV-1 and CCA-1.1 treatment on the protein level of mitotic kinases. (A) Expression band from the immunoblot assays on mitotic proteins from PGV-1 and CCA-1.1 cell lysate. Quantification of the relative protein expression levels of (B) p-Aurora A kinase, (C) p-cyclin B1, and (D) p-PLK1 after normalization with respective total protein (n = 3). Results as presented as mean ± SD from independent experiments. ANOVA was conducted for statistical analysis compared to the untreated group. CCA-1.1: chemoprevention curcumin analog-1.1, PGV-1: pentagamavunone-1, PLK1: polo-like kinase 1.

to imatinib which promotes G1 phase arrest and rapidly induces apoptosis.[22] While previous studies have indicated that G2/M arrest was resulted from CCA-1.1 treatment,[14,26] the current investigation has substantiated that CCA-1.1 selectively affected the process of mitosis, as evidenced by chromatin-based labeling. We also noticed the hyperploid cells formation in prolonged incubation due to CCA-1.1 treatment, which similarly shown in studies in aggressive breast cancer cells.[15] Thus, the cells may fail to separate or encounter mitotic catastrophe in another possible scheme, leading to death. It is noteworthy that PGV-1 also induced mitotic arrest with a higher percentage of mitotic cells (around 80%), as confirmed by both flow cytometry and chromatin staining. Given its remarkable anticancer activity compared to curcumin, this suggests that modifying the diketone structure of curcumin into a monocarbonyl likely enhances cytotoxic activity and promotes mitotic arrest in cancer cells.[29,30]

Despite being distinct by only one functional group, their molecular effects in inducing cell arrest in K-562 cells are dissimilar. CCA-1.1 tends to abrogate cell mitosis at metaphase, while PGV-1 induces prometaphase arrest,[11,31] which is also shown in this study. We observed some regulator proteins in this phase, including Aurora A, cyclin B, and PLK1, activated by phosphorylation. Among these proteins, we found that p-PLK1 was expressed in PGV-1 treatment but not in CCA-1.1 treatment. Indeed, the phosphorylation of PLK1 corresponds with the high mitotic cell population under PGV-1 treatment. PLK1 activity peaks during prometaphase and is located in the centrosome later, is activated either by phosphorylation at residue Thr210 in the kinase domain (KD) by Aurora A/BORA complex or binding with a phospho-substrate at the polo box domain (PBD). This activation leads to a conformational change (the DFG-in motif) and converts PLK1 into an active state.[32] However, after being activated in the cytoplasm, PLK1 will preferably translocate to the nucleus to phosphorylate its target due to the many phosphatases in the cytoplasm. PLK1 must diffuse freely between the cytoplasm and nucleus, and this dynamic localization is required to function properly during G2/M.[33] PGV-1 may interrupt this dynamic; therefore, even if the PLK1 is phosphorylated, it cannot function properly, disrupting centrosome-kinetochore interactions during prometaphase and inducing mitotic arrest. In addition, the accumulation of PLK1 in the kinetochore is required to maintain the stable kinetochore-microtubule (KT-MT) attachment before metaphase.[32] The localization of PLK1 during the mitosis under PGV-1 treatment needs to be observed. Furthermore, PLK1 can phosphorylate cyclin B1 at Ser133,[34] which agrees with the finding that PGV-1 treatment led to elevated levels of phosphorylated cyclin B1 and PLK1.

As for CCA-1.1, although it dominantly induces metaphase arrest, the phosphorylation of cyclin B1 tends to present instead of being degraded by the proteasome. Moreover, some unphosphorylated cyclin B1 is also detected from the immunoblot in both treated cells. This demonstrates that CCA-1.1 arrests cells before metaphase cyclosome/APC activation. The status of CDK1 and its interaction with cyclin B should be addressed in the future. CCA-1.1-induced metaphase arrest may involve the deactivation of p-PLK1; therefore, we could not observe p-PLK1, or it could be due to the lower percentage of mitotic-arrested cells in the CCA-1.1 group. Furthermore, during metaphase, the PLK1 in the kinetochore decreases while it increases in the microtubule. In this state, SAC will be silenced, allowing chromosome segregation.[32] The mechanism of CCA-1.1-induced metaphase arrest may involve SAC activation. The active SAC has not degraded and halted p-cyclin B1, hence we still detect p-cyclin B1. A recent report shows that the abnormal presence of cyclin B1 due to extended mitotic arrest marks is one of the molecular features of the mitotic catastrophe.[35] These mechanisms can be addressed for future studies.

This mitotic catastrophe from CCA-1.1 treatment prompts either mitotic cell death or cellular senescence. Cellular senescence responds to various factors, including oxidative stress.[36] Acute ROS triggers senescence, and prolonged senescence can promote cellular death.[37] Cell senescence can result from mitotic arrest.[38] Supported by previous reports highlighting that CCA-1.1 or PGV-1 induces β-galactosidase activity, the senescence effect on K-562 cells may be correlated with the rising cellular ROS level upon the compound exposure of cancer cells.

Targeting the cell cycle, particularly mitosis, as the primary mechanism of a drug is very tricky because of the extremely short span of mitosis in a whole cell cycle. Moreover, antimitotic drugs are accompanied by side effects. Previous works established that these curcumin analogs promote good selectivity towards cancer cells.[13,25] The current study further supported that regardless of cell type, CCA-1.1 exhibits similar anticancer properties to PGV-1 in inhibiting cell proliferation by inducing cell cycle arrest, ROS, and senescence. Given the remarkable anticancer effect from CCA-1.1 in CML cells and the distinct target of mitosis compared to PGV-1, more research can be considered to determine the feasibility of future combinations with present treatments.

Conclusion

CCA-1.1 and PGV-1 have distinct molecular mechanisms in the mitotic arrest. CCA-1.1 inhibits CML K-562 cell growth through cell cycle arrest in metaphase, while PGV-1 promotes prometaphase arrest and induces phosphorylation of PLK1 and cyclin B1. Further, CCA-1.1 enhances intracellular ROS generation and mediates cellular senescence in K-562 cells.

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Author Contributions

Riris Istighfari Jenie: Funding Acquisition, Project Administration, Supervision Writing - Original Draft, Dhania Novitasari: Formal Analysis, Investigation, Writing - Original Draft. Dyaningtyas Dewi Pamungkas Putri: Formal analysis, Investigation. Ratna Asmah Susidarti: Investigation. Ikuko Nakamae: Formal Analysis. Noriko Yoneda-Kato: Resources, Supervision. Jun-ya Kato: Conceptualization, Methodology, Resources, Supervision. Edy Meiyanto: Conceptualization, Methodology, Resources, Supervision.

Conflict of Interest

All authors declare no conflict of interest in this study.

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