0026-895X/87/040530-06$02.00/0

MOLECULAR PHARMACOLOGY, 32:530-535

Antiproliferative and Cytotoxic Effects of Newly Discovered Halogenated Coral Prostanoids from the Japanese Stolonifer *Clavularia viridis* on Human Myeloid Leukemia Cells in Culture

ATSUSHI HONDA, YO MORI, KAZUO IGUCHI, and YASUJI YAMADA

Departments of Biochemistry (A.H., Y.M.) and Chemistry (K.I., Y.Y.), Tokyo College of Pharmacy, 1432-1, Horinouchi, Hachioji-shi, Tokyo 192-03, Japan

Received December 15, 1986; Accepted July 3, 1987

SUMMARY

The antiproliferative and cytotoxic activities of newly discovered halogenated coral prostanoids (chlorovulone, bromovulone, and iodovulone) from the Japanese stolonifer *Clavularia viridis* and their related compounds were determined in HL-60 cells in culture. The order of antiproliferative and cytotoxic activities of naturally occurring marine prostanoids against HL-60 cells was chlorovulone I > bromovulone I = iodovulone I > clavulone I or II > prostaglandin A_2. The IC_{50} (concentrations required to inhibit cell growth by 50%) value (0.03 μM (0.01 μg/ml)) and cytotoxic effects (> 0.3 μM (0.1 μg/ml)) of chlorovulone I were about 200 and 100 times stronger than those of prostaglandin A_2, respectively, on the molar basis. From our data on the structure-activity relationship of the halogenated coral prostanoids and the related compounds, we elucidated that 1) the alkylidencyclopentenone structure in these prostanoids was essential for the antiproliferative and cytotoxic activities against HL-60 cells and the introduction of halogen function at C-10 position in the structure enhanced the activities (C1=F>Br=I>H); 2) the stereospecificity of the 12-hydroxyl group in the chlorovulone molecule was not required for the activities; 3) the presence of dienone (C_{5-6} and C_{7-8}) in the structure potentiated the activities. Bivariate DNA/bromodeoxyuridine analysis with a flow cytometer showed that chlorovulone I transiently arrested the cell cycle progression from G_1 to S after 24-hr exposure to the nontoxic concentrations (0.03 and 0.09 μM) and caused the lasting blockade of leukemia cells in G_1 at the cytotoxic concentration. These results suggest that these coral prostanoids and related compounds may be a promising antileukemic agent.

Several types of prostanoids have been found in marine corals (1–4) since 1969. In 1982, a new type of marine coral prostanoids, clavulones (5, 6)[claviridenons (7, 8)], isolated from the Japanese stolonifer *Clavularia viridis* by Quoy and Gaimard, have received much attention because of their unique structural features and marked antitumor activity (9, 10). Recently, punaglandins (11), the chlorinated prostanoids, were isolated from the octocoral *Telesto riisei*, and punaglandin 3 showed a stronger antitumor activity than that of clavulone (9). Our continuing investigation on marine prostanoids from the Japanese stolonifer *C. viridis* has led to the isolation and the structural elucidation of a series of novel halogenated prostanoids; chlorovulone (chlorinated prostanoid) (12), bromovulone (brominated prostanoid) (13), and iodovulone (iodinated prostanoid) (13). Our aim is to elucidate the mode of action of these new halogenated prostanoids against HL-60 cells. Honda *et al.* (10) previously reported that clavulone arrested the cells in the G_1 phase and inhibited the cell growth of HL-60 cells by inhibiting S-phase DNA synthesis. We therefore conducted comparative studies of the antiproliferative and cytotoxic activities of these halogenated coral prostanoids and related compounds to get the information of the structure-activity relationship. In addition, the flow cytometric analysis of chlorovulone-treated HL-60 cells was made to determine the cell cycle traverse characteristics.

We report here that the newly discovered chlorinated prostanoid, chlorovulone, from *C. viridis* shows the most potent antiproliferative and cytotoxic activities against HL-60 cells in culture as judged by the data on the structure-activity relationship among these related compounds, together with the cell cycle effect of chlorovulone on HL-60 cells.

Experimental Procedures

Materials. Marine coral prostanoids (chlorovulone I, bromovulone I, iodovulone I, and clavulone I and II) isolated from the Japanese stolonifer *C. viridis* and the synthetic related compounds were supplied by Prof. Y. Yamada and Dr. K. Iguchi, Laboratory of Organic Chemistry, Tokyo College of

This work was supported in a part by Grant-in-Aid 61015098 for Cancer Research from the Ministry of Education, Science, and Culture of Japan and by the Science Research Promotion Fund of Japan Private School Promotion Foundation.

ABBREVIATIONS: PBS, phosphate-buffered saline; BrdUrd, bromodeoxyuridine; PI, propidium iodide; FITC, fluorescein isothiocyanate.

Pharmacy. (−)-Chlorovulone II was synthesized by Dr. H. Nagaoka *et al.* (14). 15-(*S*)-prostaglandin A_2 and 15-(*R*)-prostaglandin A_2 were generous gifts from Dr. J. E. Pike (Upjohn, Kalamazoo, MI).

The following commercial materials were used: fetal calf serum (mycoplasma- and virus-free) from Boehringer Mannheim GmbH, FRG, medium RPMI 1640 from Grand Island Biological Co. (New York); streptomycin sulfate from Meiji Seika (Tokyo); penicillin from Banyu Pharmaceutical (Tokyo); trypan blue from Tokyo Chemical Industry (Tokyo); BrdUrd, propidium iodide, and Tween 20 from Sigma Chemical (St. Louis, MO); antibromodeoxyuridine monoclonal antibody and FITC-antimouse immunoglobulin G from Becton Dickinson Monoclonal Center, Mountain View, CA. All other reagents were of the highest grade commercially available.

Cell and cell culture. A human promyelocytic leukemia cell line, HL-60, was established by Collins *et al.* (15) from the peripheral blood of a patient with acute promyelocytic leukemia. The HL-60 cells were kindly supplied by Drs. M. Miwa and M. Kato, National Cancer Center Research Institute, Tokyo. The cells were cultured in suspension in RPMI 1640 medium supplemented with 10% (vol/vol) fetal calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml) at 37°C for 72 hr in a humidified atmosphere of 5% CO_2/95% O_2 as described previously (10, 16). The cells were inoculated at about 3.7×10^5 cells/ml in T flasks (20 ml culture medium). Halogenated coral prostanoids or their related compounds dissolved in ethyl alcohol were added a single time to the culture medium to keep the final ethyl alcohol concentration less than 0.2%, and the same amount of vehicle was added to the control culture.

Growth inhibition studies. The growth inhibition studies of halogenated coral prostanoids or their related compounds on HL-60 cells were performed essentially as described previously (10). HL-60 cells (about 3.7×10^5 cells/ml) were cultured in T flasks (20 ml RPMI 1640 medium supplemented with 10% fetal calf serum) with or without halogenated prostanoids or the related compounds for 72 hr at 37°C in a humidified atmosphere as described above. The viable cells were counted with a hemocytometer by the trypan blue dye exclusion method after culture of the cells with or without halogenated prostanoids or the related compounds for 24, 48, and 72 hr, respectively.

Cell staining and flow cytometric analysis. Bivariate DNA/BrdUrd analysis procedures developed by Dolbeare *et al.* (17) were performed to determine the cell cycle traverse characteristics of chlorovulone-treated HL-60 cell populations.

HL-60 cells (about 3.7×10^5 cells/ml) growing exponentially in 20 ml RPMI 1640 medium supplemented with 10% fetal calf serum were continuously exposed to chlorovulone I or synthetic (−)-chlorovulone II for varying concentrations and periods of time, and after that the cells were treated with BrdUrd (final concentration 10 μM) for 30 min. The cells were washed twice with 5 ml PBS without calcium and magnesium (PBS (−)) and then fixed in cold 70% ethanol. The cells were rinsed three times with PBS (−) to remove ethanol, resuspended in 2 ml of 1.5 M HCl at room temperature for 20 min, and centrifuged at 500 × *g* for 5 min. The cell pellets were neutralized by the addition of 1 ml of 0.1 M sodium tetraborate (pH 8.5) and centrifuged at 500 × *g* for 5 min. The cell pellets were resuspended in 0.5 ml PBS (−) containing 0.5% Tween 20, 0.5% bovine serum albumin, and 5 μl anti-BrdUrd monoclonal antibody (Becton Dickinson Monoclonal Center), incubated at 20°C for 30 min, and washed twice with 5 ml cold PBS (−). The resulting cells were incubated in 0.5 ml PBS (−) containing 0.5% Tween 20, 0.5% bovine serum albumin, and 4 μl FITC-labeled antimouse IgG antibody (Becton Dickinson Monoclonal Center) at 20°C for 20 min. The cells were then washed twice with 5 ml cold PBS (−) and resuspended in 1 ml PBS (−) containing PI (5 μg/ml). After washing with cold PBS (−), the cells were ready for flow cytometric analysis.

In the flow cytometric analysis, a Fluorescence Activated Cell Sorter-IV (Becton Dickinson Immunocytometry Systems, Mountain View, CA) was used to measure simultaneously three parameters for each cell. These were forward light scatter (related to cell size), green fluorescence from FITC-conjugated antibodies, and red fluorescence from PI. During flow cytometry, cells were exited at 488 nm with argon ion laser. Red fluorescence from PI was collected through a 620 nm-long wavelength pass filter and recorded as a measure of total DNA content, and green fluorescence from fluorescein was collected through a 530-nm bandpass filter and recorded as a measure of the amount of incorporated BrdUrd. The resulting data were accumulated to form a bivariate 64 × 64 channel distribution showing the distribution of DNA (red fluorescence) and BrdUrd (green fluorescence) among the cells of the population. Assignment of various phases (G_1, S, and G_2-M) with the flow cytometer and calculations of percentage of cells in various phases of the cell cycle were made by using a Consort 30 (HP 310) computer system (Becton Dickinson Immunocytometry Systems).

Results

Growth inhibition and cytotoxic effects of HL-60 cells by natural halogenated prostanoids and synthetic related compounds. Table 1 summarizes the antiproliferative and cytotoxic activities of newly discovered halogenated coral prostanoids from the Japanese stolonifer *C. viridis* on HL-60 cells *in vitro*. (Fig. 1 shows the structures of a series of new halogenated coral prostanoids.) The order of antiproliferative activity expressed as IC_{50} and of cytotoxic effects of the naturally occurring coral prostanoids against HL-60 cells was chlorovulone I > bromovulone I = iodovulone I > clavulone I. Chlorovulone I showed the strongest antiproliferative (IC_{50} = 0.03 μM (0.01 μg/ml)) and cytotoxic activities (>0.3 μM (0.1 μg/ml)) against HL-60 cells among the coral prostanoids, and the antiproliferative activity (IC_{50} value) is about 13 times stronger than that of clavulone I on the molar basis. The IC_{50} values of bromovulone I and iodovulone I were almost even and were about seven times stronger than that of clavulone I. These results suggest that chlorinated coral prostanoids such as chlorovulone I and punaglandin 3 (9) have the most potent anti-

TABLE 1

Inhibitory effects of marine coral prostanoids on cell growth of HL-60 cells

Coral prostanoids	IC_{50}[a]	Cytotoxic effect[a]
	μM (μg/ml)	
Chlorovulone I	0.03 (0.01)	>0.3 (0.1)
Bromovulone I	0.06 (0.025)	>0.9 (0.4)
Iodovulone I	0.06 (0.03)	>0.9 (0.4)
Clavulone I	0.4 (0.2)	>1.0 (0.5)

[a] Values determined as described in Ref. 10.

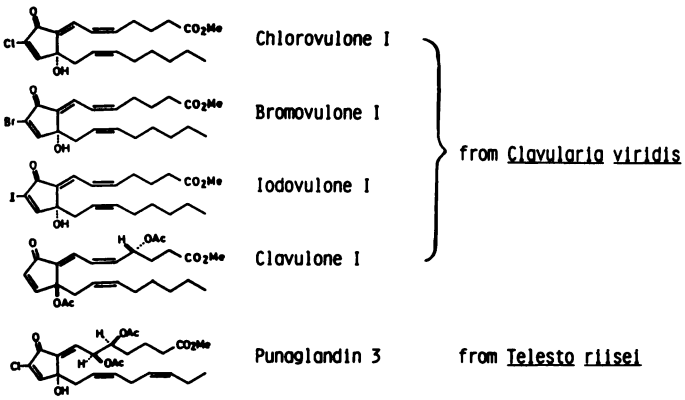

Fig. 1. The structures of a series of new halogenated marine coral prostanoids. The structures of chlorovulone I (12, 14), bromovulone I (13), iodovulone I (13), and clavulone I (5, 6) were determined. The structures of punaglandins were first proposed by Baker *et al.* (11), but the structures were revised by Nagaoka *et al.* (20) and Suzuki *et al.* (21). The revised structure of punaglandin 3 is shown in Fig. 1. —OAc = —$OCOCH_3$, —CO_2Me = —CO_2CH_3.

TABLE 2

Inhibitory effects of coral prostanoids and their related compounds on the cell growth of HL-60 cells

Compounds	IC_{50}[a]	Cytotoxic effect[a]
	μM (*μg/ml*)	
(−)-Chlorovulone II	0.03 (0.01)	>0.3 (0.1)
10-Chloro-12-O-desacetylclavulone II	0.03 (0.015)	>0.3 (0.15)
10-Fluoro-12-O-desacetylclavulone II	0.03 (0.013)	>0.3 (0.15)
Clavulone II	0.4 (0.2)	>1.0 (0.5)
10,11-Dihydroclavulone II	2.2 (1.0)	>11.1 (5.0)
15-(*S*)-Prostaglandin A_2	6.0 (2.0)	>30.0 (10.0)
15-(*R*)-Prostaglandin A_2	6.0 (2.0)	>30.0 (10.0)

proliferative activity against leukemia cells among coral prostanoids that have been found so far.

Table 2 shows the antiproliferative and cytotoxic activities of synthetic (−)-chlorovulone II, synthetic clavulone II analogs, and 15-(*S*)- and 15-(*R*)-prostaglandin A_2 against HL-60 cells *in vitro*. (Fig. 2 shows the structures of synthetic related compounds.) The IC_{50} value and cytotoxic effects of synthetic (−)-chlorovulone II (enantiomer of natural chlorovulone II) were almost the same compared with those of the natural chlorovulone I, suggesting that stereospecificity of the 12-hydroxyl group in the chlorovulone molecule is not required for the inhibition of HL-60 cell proliferation. The IC_{50} values and cytotoxic effects of synthetic halogenated clavulone II analogs, 10-chloro-12-O-desacetylclavulone II and 10-fluoro-12-O-desacetylclavulone II, were nearly equal to those of the natural chlorovulone I, suggesting that the introduction of chlorine or fluorine function at C-10 position in the natural clavulone II molecule enhances the antiproliferative and cytotoxic activities against HL-60 cells. The values of IC_{50} and cytotoxic effects of 10,11-dihydroclavulone II increased to about 5.5 times and about 11 times, respectively, compared with those of the natural clavulone II. This result suggests that the presence of a reactive α,β-unsaturated carbonyl group ($C_{10\text{-}11}$) in the cyclopentane ring of clavulone II is essential for the antiproliferative and cytotoxic activities against HL-60 cells. The IC_{50} values and cytotoxic effects of 15-(*S*)-prostaglandin A_2 were almost the same compared with those of 15-(*R*)-prostaglandin A_2, and these prostaglandin A_2 values were about three times greater than those of 10,11-dihydroclavulone II. These results suggest that *S* absolute configuration of the 15-hydroxy group is not required for the inhibition of HL-60 cell proliferation and that the presence of dienone ($C_{5\text{-}6}$ and $C_{7\text{-}8}$) of 10,11-dihydroclavulone II partly contributes to the inhibition of HL-60 cell growth. Thus, the antiproliferative (IC_{50} value) and cytotoxic effects of chlorovulone I against HL-60 cells are about 200 and 100 times stronger than those of prostaglandin A_2, respectively, on the molar basis.

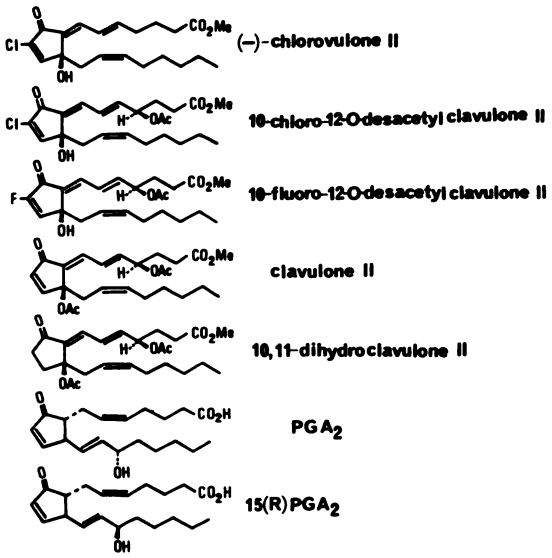

Fig. 2. The structures of coral prostanoids and their related compounds. PGA_2, prostaglandin A_2. —OAc = —$OCOCH_3$, —CO_2Me = —CO_2CH_3.

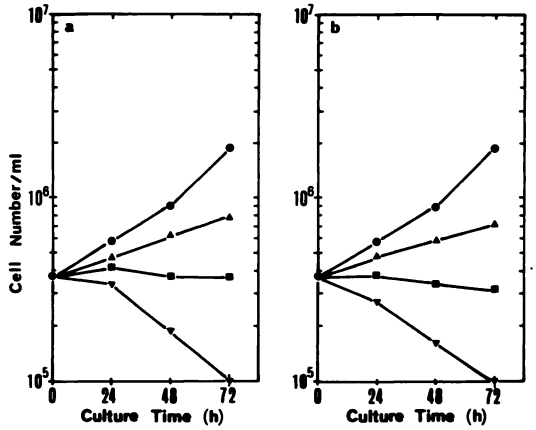

Fig. 3. Effects of natural (+)-chlorovulone I (a) and synthetic (−)-chlorovulone II (b) on HL-60 cell growth. The procedures for kinetics of growth inhibition of HL-60 cells by the natural or the synthetic chlorovulone are described in the text. ●—● control, ▲—▲ 0.03 *μM* (0.01 *μg/ml*), ■—■ 0.09 *μM* (0.03 *μg/ml*), ▼—▼ 0.15 *μM* (0.05 *μg/ml*).

Kinetics of growth inhibition of HL-60 cells by natural (+)-chlorovulone I and synthetic (−)-chlorovulone II. Figure 3 shows the growth curve of HL-60 cells in the presence or absence of chlorovulone I (Fig. 3a) or synthetic (−)-chlorovulone II (Fig. 3b). The kinetics of inhibition of growth by the natural chlorovulone I of HL-60 cells were almost the same compared with those by the synthetic (−)-chlorovulone II. Untreated HL-60 cells grew exponentially with a generation time of approximately 35 hr until 72-hr culture periods. When HL-60 cells were grown with various concentrations of chloro-

vulone I or synthetic (−)-chlorovulone II for 72 hr, the two compounds caused the decrease in the cell growth of HL-60 cells in a dose-dependent and time-dependent manner. Chlorovulone I or synthetic (−)-chlorovulone II decreased the growth rate of HL-60 cells to half the level of the control cells at about 0.03 μM (0.01 μg/ml), and inhibited the growth almost completely over 0.09 μM (0.03 μg/ml). At higher concentrations of chlorovulone I or synthetic (−)-chlorovulone II such as 0.15 μM (0.05 μg/ml) and 0.3 μM (0.1 μg/ml), it showed significant cytotoxic activities against HL-60 cells as judged by the trypan blue dye exclusion method.

To determine whether the effect of chlorovulone I was reversible, we discarded the medium containing chlorovulone I at 0.09 μM (0.03 μg/ml) after culture for 72 hr, washed the cells by centrifugation, and recultured them in 10% (vol/vol) fetal calf serum-RPMI 1640 medium without chlorovulone I for further 3–4 day culture. These chlorovulone I-pretreated cells for 72 hr grew gradually, and the cell number increased gradually for the further culture (data not shown). This indicates that the cytostatic effects of chlorovulone I are reversible.

Effects of chlorovulone I on cell cycle progression of HL-60 cells. We determined the cell cycle traverse characteristics of chlorovulone I-treated HL-60 cell populations by bivariate DNA/BrdUrd analysis.

Figure 4 shows bivariate DNA/BrdUrd distributions measured for HL-60 cells at 24-hr intervals with or without various concentrations of chlorovulone I (as indicated by Fig. 3). The distribution of the untreated exponentially growing control cells consisted of three cell populations: G_1-phase- (51.0%) and G_2-M-phase-cells (9.0%) that have low BrdUrd-linked fluorescence and the S-phase cells (40.0%) that have high BrdUrd-linked fluorescence. In contrast, the cells after 24-hr exposure to increasing concentrations of chlorovulone I from 0.03 (0.01 μg/ml) to 0.15 μM (0.05 μg/ml) showed an increased proportion of G_1-phase cells (65.5–70.0%) with markedly decreased numbers of S-phase cells (24.1–9.3%).

Figure 5 shows the time of changes in bivariate DNA/BrdUrd distributions measured for HL-60 cells at various culture periods with or without various concentrations of chlorovulone I (as indicated in Fig. 3). The cell cycle phase distributions of the untreated exponentially growing control cells did not significantly change during 72-hr culture periods: G_1-phase cells (about 50–40%), S-phase cells (about 40–50%), and G_2-M-phase cells (about 10%) (Fig. 5a). In contrast, the distributions of the cells after 24-hr exposure to 0.03 μM (0.01 μg/ml) and 0.09 μM (0.03 μg/ml) of chlorovulone I showed a maximum increase in proportion of G_1-phase cells (about 60–70%) with a corresponding decreased number of S-phase cells (about 24–10%), although at these doses the cell growth was partly or completely inhibited as indicated by Fig. 3, but after that the cell cycle phase distributions of the treated cells were nearly close to those of the untreated control level during 48–72 hr culture (Fig. 5b and c). At higher doses (0.15 μM (0.05 μg/ml) chlorovulone I), the cell growth stopped completely as indicated in Fig. 3, the proportion of G_1-phase cells increased to about 76% and that of S-phase cells decreased to about 10%, and G_2-M-phase cells were about 10–20% during 72-hr culture periods (Fig. 5d). These results indicate that chlorovulone I arrests the cell cycle progression from G_1 to S after 24-hr exposure to the nontoxic concentrations (0.03 μM (0.01 μg/ml) and 0.09 μM (0.03 μg/ml)), but the effect of chlorovulone I is transient and recovers by 48 h to the zero time control and that the only

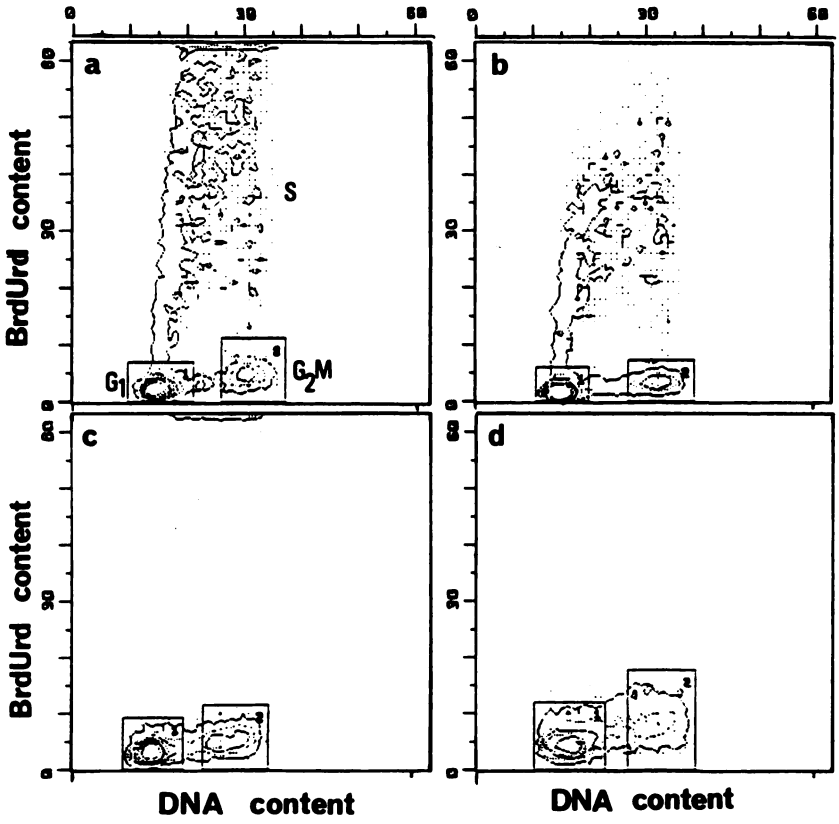

Fig. 4. Bivariate DNA/BrdUrd distributions measured for exponentially growing HL-60 cells at 24-hr intervals with or without natural chlorovulone I. Abscissas and ordinates represent univariate frequency distributions for total DNA content (red fluorescence) and amount of incorporated BrdUrd (green fluorescence), respectively. The experimental procedures for cell staining and bivariate DNA/BrdUrd analysis with a flow cytometer are described in the text; *a*, control; *b*, 0.03 μM (0.01 μg/ml); *c*, 0.09 μM (0.03 μg/ml); *d*, 0.15 μM (0.05 μg/ml). The number of viable cells at each 24-hr treatment is shown in Fig. 3a.

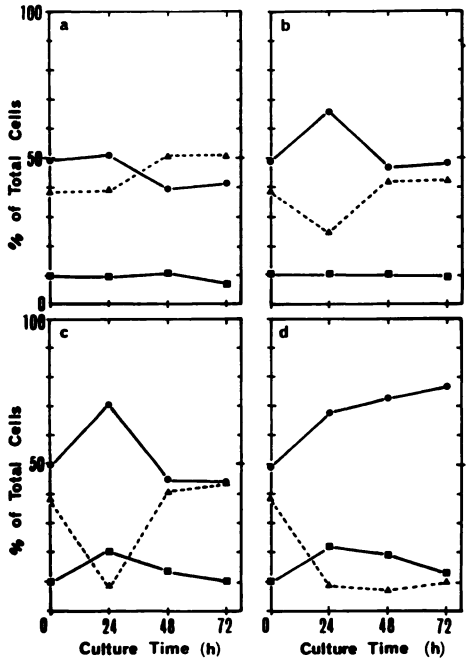

Fig. 5. Time- and dose-dependent changes in the cell distributions of HL-60 cells during continuous exposure to natural chlorovulone I. These results were obtained by quantitative analysis of the bivariate DNA/BrdUrd distributions such as those shown in Fig. 4 at various culture times and are the means of two independent experiments. All values are within ± 10% of the mean; *a*, control; *b*, 0.03 μM (0.01 μg/ml); *c*, 0.09 μM (0.03 μg/ml); *d*, 0.15 μM (0.05 μg/ml). ●—● G_1-phase cells, ▲- - -▲ S-phase cells, ■—■ G_2-M-phase cells. The experimental procedures for bivariate DNA/BrdUrd analysis with a flow cytometer are described in the text.

lasting blockade of cell cycle in G_1 appears at the 0.15 μM (0.05 μg/ml) concentration of chlorovulone I. Also, almost the same results of cell cycle progression of HL-60 cells as described in the case of the natural chlorovulone I (Fig. 5) were obtained in the synthetic (−)-chlorovulone II (data not shown).

Discussion

The discoveries of new types of marine coral prostanoids with antitumor activity (clavulone (5, 6, 9, 10, 18, 19), chlorovulone (12, 14), bromovulone (13), and iodovulone (13) from the Japanese stronifer *C. viridis* in Okinawa and punaglandin (9, 11, 20, 21) from the octocoral *T. riisei* in Hawaii), have led to interest in structural, biological, and comparative biochemical aspects.

From our data on the structure-activity relationship of the halogenated coral prostanoids and the related compounds (Tables 1 and 2), we elucidated that 1) the order of antiproliferative and cytotoxic activities of naturally occurring marine prostanoids against HL-60 cells was chlorovulone I > bromovulone I = iodovulone I > clavulone I or II > prostaglandin A_2, 2) the stereospecificity of the 12-hydroxyl group in the chlorovulone molecule was not required for the antiproliferative and cytotoxic activities, 3) the introduction of halogen (Cl or F) function at C-10 position potentiated the antiproliferative and cytotoxic activities (Cl = F > Br = I > H), 4) a double bond in C_{10-11} in the cyclopentane ring was essential for the antiproliferative and cytotoxic activities [this result is in good agreement with the findings by Honn and Marnett (22)], 5) the presence of dienone (C_{5-6} and C_{7-8}) potentiated the antiproliferative and cytotoxic activities, and 6) *S* absolute configuration of the 15-hydroxyl group in prostaglandin A_2 was not required for the antiproliferative and cytotoxic activities (this result is in good agreement with the findings of the B16 amelanotic melanoma (22)). These results that alkylidencyclopentenone structure is essential for the antiproliferative and cytotoxic activities against HL-60 cells are consistent with the findings that alkylidencyclopentenone prostaglandin derivatives such as Δ^7-prostaglandin A_1 and Δ^{12}-prostaglandin J_2 have more potent antitumor activity than their parent compounds such as prostaglandin A_1 and prostaglandin D_2 (23–25). Thus, in contrast to mammalial occurring prostanoids, the new types of naturally occurring marine coral prostanoids, such as clavulone (5, 6), chlorovulone (12), bromovulone (13), iodovulone (13), and punaglandin (11), should be classified as a new category of prostanoids because of the unique structural features and the markedly potent cytostatic and cytotoxic activities.

From previous reports (10, 26–28), it is hypothesized that the inhibition mechanism of the cell growth by the coral prostanoids, alkylidencyclopentenone prostaglandins (Δ^7-prostaglandin A_1 and Δ^{12}-prostaglandin J_2), and prostaglandins A_1, A_2, and D_2 can be explained by the mode of action as G_1 blocker of the cell cycle, although the study should be extended to other malignant tumor forms before a generalization on the drug action can be concluded. Then we analyzed the cell cycle traverse characteristics of chlorovulone I-treated HL-60 cell population by the bivariate DNA/BrdUrd analysis. Figure 5 shows that the effect of chlorovulone I on arresting the HL-60 cell cycle progression from G_1 to S appears at 24 hr at nontoxic concentrations (0.03 μM (0.01 μg/ml) and 0.09 μM (0.03 μg/ml)) but recovers at 48 and 72 hr to the zero time control. The transient effect of chlorovulone at nontoxic concentrations may be due to its instability, or inactivation, in the presence of cells. However, at this stage it is difficult to state the accurate biological lifetime and metabolic fate of chlorovulone and related compounds in the presence of cells because the radiolabeled synthetic compounds are unavailable. The only concentration that demonstrated the true block of cell cycle in G_1 was at the 0.15 μM (0.05 μg/ml) concentration (Fig. 5d). However, at the 0.15 μM concentration of chlorovulone I, the cytotoxic effects appear as judged by the trypan blue dye exclusion methods and the results shown in Fig. 3. Thus the lasting G_1 block of chlorovulone at the 0.15 μM concentration may be due to dying cells. Chlorovulone- and clavulone-induced lethal effects on the cells may be directly related to the block of S-phase DNA synthesis (Figs. 4 and 5 and Ref. 10); however, the mechanism by which chlorovulone and clavulone cause the cell cytotoxicity is still unclear. Recently, Narumiya *et al.* (29, 30) reported that cyclopentenone prostaglandins such as prostaglandin A and J were transported actively and selectively into cultured cells by a carrier-mediated process and accumulated in intracellular organella, including nuclei.

The potent antiproliferative and cytotoxic action of chlorovulone and clavulone (10) suggests a possible important drug administration to leukemia in that the drug combinations of these coral prostanoids with other antileukemic agents (metaphase arrest or G_2 blocker) are likely to cause synergistic kill of leukemia cells. We believe that these coral prostanoids and related compounds may be a promising antileukemic agent in

the therapy of human leukemia if we use a leukemia cell-specific drug delivery system.

Acknowledgments

We are grateful to Professor Philip Needleman (Washington University School of Medicine) for helpful suggestions and encouragement. We thank Drs. Hiroyuki Kikuchi and Koichi Ina (Fujisawa Pharmaceutical) for their generous assistance with the flow cytometric analysis. We also thank Misses Chitose Sato and Chikako Arai (Tokyo College of Pharmacy) for their technical assistance in part of this work.

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Send reprint requests to: Dr. A. Honda, Department of Biochemistry, Tokyo College of Pharmacy, 1432-1, Horinouchi, Hachioji-shi, Tokyo 192-03, Japan