Cytotoxicity of Platelet Activating Factor and Related Alkyl-Phospholipid Analogs in Human Leukemia Cells, Polymorphonuclear Neutrophils, and Skin Fibroblasts

By Dennis R. Hoffman, Joseph Hajdu, and Fred Snyder

A series of 11 alkyl-phospholipid analogs, structurally related to platelet activating factor (L-PAF), were analyzed for cytotoxic activity in human leukemic (HL-60) cells, human polymorphonuclear neutrophils, and Detroit 551 human skin fibroblasts. The order of selectiveness of the analogs in their cytotoxic response toward HL-60 cells in comparison to neutrophils is 1-alkyl-2-acetamide-GPC \( > \) 1-alkyl-2-methoxy-GPC \( > \) D-PAF \( > \) 1-acyl-2-lyso-GPC \( > \) 1-alkyl-2-lyso-GPC \( > \) L-PAF. A time-sequenced progression of events caused by the most potent cytotoxic alkyl-phospholipid analogs was characterized by (a) a rapid decrease in the cellular uptake and incorporation of \( ^3H \)-thymidine into DNA that was detectable 4 hr after exposure to the analog, (b) a release of lactate dehydrogenase activity into the media at 8 hr after exposure, and (c) a decrease in cell number due to cell death that begins at 12 hr after exposure. Treatment of HL-60 cells with 1-alkyl-2-methoxy-GPC for 1 hr destroyed 40% of the cells after a subsequent 24-hr incubation period. The varied biologic activities of L-PAF, including how it affects serotonin release from platelets, blood pressure in rats, and cytotoxic responses in normal and leukemic cells, are discussed in relation to its D-enantiomer, 3-alkyl-2-acetyl-GPC, and the 2-acetamide analog. This report characterizes the kinetic events of the cellular responses in both normal and HL-60 cells in relation to the antineoplastic activities of unnatural ether-linked phospholipid analogs that are structurally related to L-PAF.

Certain analogs of 1-alkyl-2-lyso-sn-glycero-3-phosphocholine (1-alkyl-2-lyso-GPC) that have alkyl substituents at the sn-2-position have been shown to possess antineoplastic activity. The most potent cytotoxic phospholipid derivative reported so far is 1-octadecyl-2-methoxy-GPC. The 2-methoxy analog destroys various types of neoplastic cells at doses considerably lower than are toxic to normal cells. The structural similarities of the biologically active phospholipid, 1-alkyl-2-acetyl-GPC or platelet activating factor (L-PAF), to the antineoplastic unnatural alkyl-phospholipid analogs prompted our investigation of the cytotoxic effects of L-PAF and closely related analogs in human promyelocytic leukemia cells (HL-60), human polymorphonuclear neutrophils (PMNs), and Detroit 551 human skin fibroblasts. Three chemical structural features appear to be essential for effective antineoplastic activity of the unnatural phospholipid analogs of PAF: (1) an alkyl moiety at the 1-position, (2) an apparent nonmetabolizable group at the 2-position, and (3) phosphocholine at the 3-position of sn-glycerol.

Two earlier reports that dealt with induction of differentiation and the cytotoxic action of the 2-methoxy derivative in HL-60 cells utilized media supplemented with fetal bovine serum in the cultures. However, recent results have shown that elevated levels of serum diminish the cytotoxicity of alkyl-phospholipid analogs in cultures of human leukemic cells. Moreover, the presence of an acetylhydrolase, a specific esterase for 1-alkyl-2-acetyl-GPC, in serum would substantially decrease the level of L-PAF and its cytotoxic activity in serum-supplemented culture systems. Therefore, the chemically defined media used in our experiments eliminates the influence of undetermined quantities of lipids, proteins (including enzymes), and hormones in serum in the interpretation of the cytotoxic response.

Kinetic details of the responses of normal and cancerous cells to specific alkyl-phospholipid analogs are presented in this report as a basis for further investigations to elucidate the mechanism of cytotoxic action of these novel antineoplastic lipids. Furthermore, all of the phospholipid analogs tested for their cytotoxic activity in the present study have been previously compared in an investigation of their hypotensive activity in rats and their ability to induce serotonin release from rabbit platelets, two characteristic responses elicited by L-PAF. Our studies have also identified the potent selective antineoplastic response of two new analogs of platelet activating factor—1-alkyl-2-acetamide-GPC and D-PAF. The cytotoxic-
ity of the biologically active phospholipid, L-PAF, has been documented for the first time in cultured normal and neoplastic cells.

MATERIALS AND METHODS

Alkyl-phospholipids

1-Hexadecyl-2-methoxy-GPC (1-alkyl-2-methoxy-GPC) was purchased from R. Berchtold, Biochemisches Labor, Mattenhofstrasse 34, 3007 Bern, Switzerland. L-PAF (derived from beef heart plasmalogen) and 1-acyl-2-lyso-GPC (hydrolyzed egg yolk phosphatidylcholine) were purchased from Sigma Chemical Co., St. Louis, MO. Synthesis of the compounds containing an amide linkage at the 2-position, 1-octadecyl-2-stearamide-GPC and 1-octadecyl-2-acetamide-GPC, has been previously described.\(^{14,15}\) 1-Alkyl-2-ethoxy-GPC was a gift from Dr. R. L. Wykle, Bowman Gray School of Medicine, Winston-Salem, NC. A racemic mixture of hexadecyl-octadecenoyl-GPC (dt-alkyl-acyl-GPC; from R. Berchtold) was treated twice with phospholipase A\(_2\) (\textit{Phospholipaghus hannah} from Sigma Chemical Co.) to convert the L-isomer to the lyso form.\(^{5}\) octadecenoyl-GPC (DL-alkyl-acyl-GPC; from R. Berchtold) was purified by thin-layer chromatography after two phospholipase A\(_2\) treatments\(^{16}\) of a racemic mixture of DL-PAF purchased from American Type Culture Collection (Rockville, MD) and the leukocytes were then concentrated in the suspension by "pressure-forced" pipetting before cell enumeration and biochemical analysis. Fibroblasts were scraped from culture wells with a Teflon policeman, and aliquots of the cell suspension were transferred to 12 x 75 mm tubes for analysis.

Cytotoxic Tests

Three established methods were utilized to assess the cytotoxic potency of the individual alkyl-phospholipid derivatives. Cell growth was determined by enumeration of viable cells\(^2\) (those that retain a cell membrane permeability barrier against trypan blue dye intrusion). Release of cytosolic lactate dehydrogenase (LDH) activity into the media\(^23\) (indicating rupture\(^24\) or increased leakiness\(^25\) of the cell membrane) was assayed by the spectrophotometric method of Kornberg.\(^{26}\) Specific activity of the conversion of pyruvate to lactate is expressed as nmole/min/ml cell-free media.\(^2\) A third criterion of the cytotoxic activity of the analogs was based on the incorporation of \(^{3}\)H]thymidine (\(^{3}\)HTdR) into cellular DNA. After a 4-hr incubation of 500 \(\mu\)l of cell suspension with 0.5 \(\mu\)Ci (75 pmole) [methyl-\(^{3}\)H]-thymidine (New England Nuclear, Boston, MA, specific activity 6.7 Ci/m mole), an aliquot (20 \(\mu\)l) was applied to a 23-mm 3MM filter paper disk (Whatman Inc., Clifton, NJ), dried, then batched 3 times in 100 ml 5% trichloroacetic acid and once in 100 ml of water. Radioactivity bound to the disks represents the amount of \(^{3}\)HTdR incorporated into DNA.\(^{28}\) Cellular uptake of \(^{3}\)HTdR was determined after 3 washes (5 ml PBS) of the HL-60 cells from a 400-\(\mu\)l aliquot of the incubation mixture.

RESULTS

The unnatural alkyl-phospholipid derivatives tested in the HL-60 cell system can be categorized according to three types of cytotoxic responses: (1) those that are highly cytotoxic (D-PAF, 2-methoxy, and 2-acetamide analogs), (2) those of intermediate cytotoxicity (2-lyso analogs and L-PAF), and (3) those possessing little or no cytotoxicity (DL-alkyl-2-acyl-GPC and the 2-stearamide derivative) (see Figs. 1 and 2). An index of the cytotoxic potency of each phospholipid analog tested was established by determining the concentration of the lipid required to obtain (1) a 50% inhibition of cell growth, (2) a 50% reduction in \(^{3}\)HTdR incorporation into DNA, and (3) an arbitrary twofold increase of LDH activity in media as compared to controls (Table 1). The most potent analog was the 2-acetamide derivative, which required a concentration of only 0.32 \(\mu\)M to significantly inhibit HL-60 cell viability during a 24-hr incubation. DL-Alkyl-2-acyl-GPC and the 2-stearamide analog were found to be relatively non-toxic, and in some experiments, they actually stimulated both cell growth and \(^{3}\)HTdR incorporation into DNA (data not shown).

Suitable antineoplastic agents must exhibit a selec-
Cytotoxicity of Alkyl-Phospholipid Analogs

Fig. 1. Inhibition of HL-60 cell growth by alkyl-phospholipid analogs. Various concentrations of analogs, as indicated on the abscissa, were added to cultures of HL-60 leukemia cells (10^5/ml) 24 hr before cell viability was determined. See Materials and Methods for details on specific chemical structures of the compounds tested. Values are means from at least three separate experiments.

Fig. 2. Release of lactate dehydrogenase (LDH) from HL-60 cells into the media after treatment with alkyl-phospholipid analogs. Experimental samples are the same as those that were used to obtain the data in Fig. 1. The value of 100% for LDH activity, as measured spectrophotometrically in cell-free growth media, was 5.18 ± 0.93 nmole/min/ml (n = 18).

ductive killing of tumor cells compared to normal cells, and the 2-methoxy analog has been previously shown to be effective in this regard. Therefore, we also evaluated the cytotoxic actions of several representative phospholipid analogs in two normal human cell types—PMNs and skin fibroblasts. PMNs, isolated from human blood, were much more resistant to destruction by the analogs than the HL-60 cells (Table 2). D-PAF, the isomeric enantiomer of L-PAF, was the most toxic analog tested with PMNs, yet it was still four times less toxic than against the HL-60 cells. The 2-methoxy (78 μM) and 2-acetamide (30 μM) derivatives were 52- and 94-fold, respectfully, less cytotoxic (in terms of cell death) to PMNs than HL-60 cells. A second normal cell line, Detroit 551 human skin fibroblasts, was also used to index the cytotoxic actions of the alkyl-phospholipids (Table 3). At 20, 65, and 35 μM concentrations, the 2-acetamide, 2-methoxy, and D-PAF analogs were 62-, 43-, and 13-fold less toxic, respectively, to fibroblasts as compared to HL-60 cells. Table 4 shows that the 1-alkyl-2-acetamide-GPC possesses the highest selectivity of all phospholipid analogs tested in its cytotoxic response toward HL-60 cells as compared to normal cells (PMNs and skin fibroblasts).

Table 1. Cytotoxicity of Phospholipid Analogs in HL-60 Cells

<table>
<thead>
<tr>
<th>Phospholipid Species</th>
<th>Cell Number (IC_{50}^\text{*})</th>
<th>[^{3}H\text{TdR} \text{Incorporation into DNA (IC}_{50}^\text{*})</th>
<th>LDH (2 X Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Alkyl-2-acetamide-GPC (6)</td>
<td>0.32 ± 0.10†</td>
<td>1.01 ± 0.02</td>
<td>0.64 ± 0.08</td>
</tr>
<tr>
<td>1-Alkyl-2-methoxy-GPC (8)</td>
<td>1.50 ± 0.31</td>
<td>0.94 ± 0.06</td>
<td>1.15 ± 0.11</td>
</tr>
<tr>
<td>D-PAF (3)</td>
<td>2.65 ± 0.85</td>
<td>1.75 ± 0.45</td>
<td>1.53 ± 0.33</td>
</tr>
<tr>
<td>1-Alkyl-2-lyso-GPC (3)</td>
<td>11.7 ± 1.1</td>
<td>12.0 ± 1.9</td>
<td>10.0 ± 2.45</td>
</tr>
<tr>
<td>L-PAF (4)</td>
<td>13.3 ± 1.0</td>
<td>13.4 ± 1.7</td>
<td>9.4 ± 1.7</td>
</tr>
<tr>
<td>1-Acyl-2-lyso-GPC (6)</td>
<td>16.0 ± 2.3</td>
<td>16.5 ± 4.1</td>
<td>13.3 ± 4.4</td>
</tr>
<tr>
<td>1-Alkyl-2-ethoxy-GPC (4)</td>
<td>27.5 ± 4.1</td>
<td>14.0 ± 1.5</td>
<td>17.2 ± 0.8</td>
</tr>
<tr>
<td>D-3-Alkyl-2-lyso-GPC (3)</td>
<td>76 ± 4</td>
<td>67 ± 11</td>
<td>58 ± 10</td>
</tr>
<tr>
<td>D-3-Alkyl-2-acyl-GPC (3)</td>
<td>127 ± 12</td>
<td>78 ± 5</td>
<td>110 ± 9</td>
</tr>
<tr>
<td>DL-Alkyl-2-acyl-GPC (3)</td>
<td>&gt;125</td>
<td>&gt;125</td>
<td>&gt;125</td>
</tr>
<tr>
<td>1-Alkyl-2-stearamide-GPC (5)</td>
<td>&gt;140</td>
<td>&gt;140</td>
<td>&gt;140</td>
</tr>
</tbody>
</table>

The tabulated values are the means ± standard deviations. Numbers in parentheses designate the number of experiments done to obtain the mean values. See Materials and Methods section for experimental details.

*Concentration required for 50% inhibition after 24-hr incubation.
†Concentration required to produce a twofold increase in LDH activity above control values after 24-hr incubation.
‡Values are micromolar.
The order of decreasing selective tumor cytotoxicity displayed by the various phospholipid analogs tested is 1-alkyl-2-acetamide-GPC > 1-alkyl-2-methoxy-GPC > D-PAF > 1-acyl-2-lyso-GPC > 1-alkyl-2-lyso-GPC > L-PAF. These marked differences in the responses of leukemic and normal cells in culture to cytotoxic doses of 2-acetamide, 2-methoxy, and D-PAF alkyl-phospholipids indicate their potential usefulness as anticancer agents.

Cell destruction, release of LDH activity into the media, and ^{3}H TdR incorporation into DNA were used as criteria to follow the time course of the cytotoxic actions of platelet activating factor and its unnatural analogs on HL-60 cells, human PMNs, and Detroit 551 human skin fibroblasts cultured in serum-free media. A sequential progression of specific alterations in cellular events occurs in HL-60 cells exposed to the cytotoxic phospholipid analogs (Fig. 3). As early as 4 hr after treatment of the cells with 4 μM 1-alkyl-2-acetamide-GPC, release of LDH activity into the cell-free media reached control values after 24-hr incubation. 1-Alkyl-2-acetamide-GPC (2) 12.3 ± 1.1 IC_{50} Values

### Table 2. Cytotoxicity of Phospholipid Analogs in Human PMNs

<table>
<thead>
<tr>
<th>Phospholipid Species</th>
<th>Cell Number (IC_{50})</th>
<th>LDH (12x Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-PAF (3)</td>
<td>12.3 ± 1.1†</td>
<td>11.0 ± 2.8</td>
</tr>
<tr>
<td>L-PAF (2)</td>
<td>20 ± 0</td>
<td>53.8 ± 15.2</td>
</tr>
<tr>
<td>1-Alkyl-2-acetamide-GPC (3)</td>
<td>30.0 ± 2.0</td>
<td>20.7 ± 1.2</td>
</tr>
<tr>
<td>1-Alkyl-2-lyso-GPC (4)</td>
<td>42.5 ± 2.1</td>
<td>39.0 ± 4.2</td>
</tr>
<tr>
<td>1-Acyl-2-lyso-GPC (4)</td>
<td>60.1 ± 10.6</td>
<td>63.4 ± 3.4</td>
</tr>
<tr>
<td>1-Alkyl-2-methoxy-GPC (4)</td>
<td>78.3 ± 2.3</td>
<td>56.0 ± 12.1</td>
</tr>
<tr>
<td>1-Alkyl-2-stearamide-GPC (2)</td>
<td>&gt; 150</td>
<td>&gt; 150</td>
</tr>
</tbody>
</table>

The tabulated values are the means ± standard deviations. Numbers in parentheses designate the number of experiments done to obtain the mean values. See Materials and Methods section for experimental details.

* Concentration required for 50% inhibition after 24-hr incubation.
† Concentration required to produce a twofold increase in LDH activity above control values after 24-hr incubation.
‡ Values are micromolar.
§ A racemic mixture was used, 60% l-isomer and 40% d-isomer.

### Table 4. Selectivity Index of the Cytotoxic Activity of Alkyl-Phospholipid Analogs Toward HL-60 Cells in Comparison to PMNs and Skin Fibroblasts

<table>
<thead>
<tr>
<th>Phospholipid Species</th>
<th>Ratio of Cell Number IC_{50} Values*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PMNs/ Skin Fibroblasts/</td>
</tr>
<tr>
<td></td>
<td>HL-60/ HL-60</td>
</tr>
<tr>
<td>1-Alkyl-2-acetamide-GPC</td>
<td>93.8 / 62.5</td>
</tr>
<tr>
<td>1-Alkyl-2-methoxy-GPC</td>
<td>52.2 / 43.1</td>
</tr>
<tr>
<td>D-PAF</td>
<td>4.6 / 13.1</td>
</tr>
<tr>
<td>1-Acyl-2-lyso-GPC</td>
<td>3.8 / 3.5</td>
</tr>
<tr>
<td>1-Alkyl-2-lyso-GPC</td>
<td>3.6 / -</td>
</tr>
<tr>
<td>L-PAF</td>
<td>1.5 / 4.0†</td>
</tr>
</tbody>
</table>

* Ratios are derived from the IC_{50} values for viable cell numbers presented in Tables 1–3 for HL-60 cells, PMNs, and Detroit 551 skin fibroblasts.
† The IC_{50} value for cytotoxic activity of DL-PAF with skin fibroblasts was used to derive this value.

The tabulated values are the means ± standard deviations. Numbers in parentheses designate the number of experiments done to obtain the mean values. See Materials and Methods section for experimental details.

* Concentration required for 50% inhibition after 24-hr incubation.
† Concentration required to produce a twofold increase in LDH activity above control values after 24-hr incubation.
‡ Values are micromolar.
§ A racemic mixture was used, 60% l-isomer and 40% d-isomer.
2-methoxy-GPC, 3HTdR incorporation into DNA is inhibited. Next, at approximately 8 hr, the LDH activity in the media from the analog-treated cultures increases above control levels, and finally, cell death occurs about 12 hr after exposure to the unnatural alkyl-phospholipid. A decreased cellular uptake of labeled thymidine into alkyl-phospholipid-treated tumor cells has been reported previously; however, the incubation of the earlier studies were of several days' duration and, therefore, early kinetic changes were not determined. The increased potency of 1-alkyl-2-methoxy-GPC found in this investigation may be attributable to the use of serum-free growth media or to the 1-hexadecyl derivative of the methoxy analog; previous studies have utilized 1-octadecyl-2-methoxy-GPC. In our experiments, we found a decrease in the cellular uptake of 3HTdR in HL-60 cells as early as 3 hr after treatment with phospholipid analogs (data not shown); this reduced uptake preceded the decreased incorporation of 3HTdR into DNA (Fig. 3C).

Since BSA is a constituent of the serum-free media used for growth of the HL-60 cells, its effect on the cytotoxic response of the 2-methoxy analog was tested. HL-60 cells grown in serum-free media and cultured for 2 days in BSA-free medium were subjected to treatment with varying concentrations of BSA and the 2-methoxy derivative (Fig. 4). BSA concentrations up to 0.5 mg/ml increased cell growth in controls by 60% during the 24-hr incubation. Increasing concentrations of BSA diminished the cytotoxic effect of the 2-methoxy analog (2 or 12 μM), as indicated by an increase in cell number after treatment of the cells for 24 hr. However, with the lower concentration of the phospholipid analog, a BSA concentration above 0.5 mg/ml had no additional effect on the cytotoxicity. These data further document that proteins in cell culture media can greatly modify the biologic activity of phospholipids; this is probably due to the avid binding of phospholipids to proteins.

In order to determine the length of time the HL-60 cells had to be exposed to 1-alkyl-2-methoxy-GPC (12 μM) to induce cell death, the cells were washed free of the analog with PBS at the times indicated in Fig. 5. Approximately 20% of the cells exposed to the 2-methoxy analog for 30 min were killed by the end of a 24-hr incubation period, and 3HTdR incorporation into nucleic acids was inhibited by over 50% under the same conditions. After a 90-min exposure to the 2-methoxy analog, 60% of the HL-60 cells died, and 3HTdR incorporation into DNA was decreased by 80% after a 24-hr incubation. HL-60 cells surviving a 60-min or less treatment with the 2-methoxy analog and all subsequent 24-hr incubations were found to continue growing at the same rate as control cultures after a 1-day lag period, whereas cells exposed to the phospholipid analog for 90 min continued to die for the next several days (data not presented).

DISCUSSION

Three alkyl-phospholipid derivatives (2-acetamide, 2-methoxy, and D-PAF) were found to have potent selective cytotoxic action against HL-60 cells as compared to normal skin fibroblasts and neutrophils of human origin (Tables 1, 2, and 3). The 2-acetamide analog is of particular interest because it differs from L-PAF (1-alkyl-2-acetyl-GPC) only by the presence of the amide linkage at the sn-2 position instead of the

Fig. 4. Influence of varying concentrations of BSA on the cytotoxic activity of 1-alkyl-2-methoxy-GPC in HL-60 cells grown in serum-free media. HL-60 cells (10⁶/ml) grown in BSA-free media for 2 days were untreated (control, x-x) or treated with 2 μM (o-o) or 12 μM (—-—) of the 2-methoxy analog for 24 hr before determining cell viability (trypan blue dye exclusion). Values are averages of two separate experiments done in duplicate.

Fig. 5. Cytotoxic activity of 1-alkyl-2-methoxy-GPC (12 μM) in HL-60 cells as a function of the time exposed to the phospholipid analog. Treated and untreated (control) cells were washed twice with PBS at the times indicated after addition of the analog to the cell cultures. All cells were harvested for assay after a 24-hr incubation. Values are the means of determinations from two experiments.
**O-ester.** Despite the structural similarity of these two phospholipids, the acetamide analog was about 40-fold more toxic to HL-60 cells than L-PAF, and the amide analog displayed the highest degree of selective cytotoxicity toward the HL-60 cells in comparison to normal PMNs and skin fibroblasts. In contrast, other experiments related to PAF efficacy have shown that the 2-acetamide analog possesses essentially no anti-hypertensive (0.27%) or platelet activating activity (1.0%) in comparison to L-PAF (100%). Thus, it appears that the N-acetyl and O-acetyl substituents impart completely different activities to the alkyl-glycerophosphocholine species.

Others who used different tumor cell lines have reported that 1-acyl-2-lyso-GPC (lyssolecitin) had marginal tumor cytotoxic activity;\(^{30,31}\) whereas, phospholipid analogs with an alkyl group substituted for the ester group at the 1-position were more cytotoxic toward tumor cells.\(^{3,7,29,31}\) Our results show that the 1-alkyl-2-lyso-GPC and 1-acyl-2-lyso-GPC analogs are nearly equipotent in the HL-60 cell system (Figs. 1 and 2, Table 1), but 5–10-fold less effective than the three most toxic alkyl-phospholipids.

HL-60 cells are capable of metabolizing L-PAF by the combined actions of acetylatediolase and acyltransferase to form 1-alkyl-2-acyl-GPC via a lyso intermediate.\(^{32}\) Conversion of 1-alkyl-2-acyl-GPC (L-PAF) to 1-alkyl-2-lyso-GPC\(^{10}\) probably explains the similar cytotoxic activities of these two compounds in HL-60 cell cultures; both agents had IC\(_50\) values between 9 and 14 \(\mu M\) (Table 1). Subsequent metabolism of the lyso analog to the less cytotoxic 1-alkyl-2-acyl-GPC by an acyltransferase could account for the intermediate toxicity of the 2-lyso derivatives.

1-Alkyl-2-ethoxy-GPC was considerably less toxic than the 2-methoxy analog in HL-60 cells (Table 1). It is of interest that at very high concentrations, the 2-ethoxy derivative can induce biologic actions in rabbit neutrophils similar to L-PAF;\(^{33}\) however, the 2-ethoxy analog is only 2% as active as L-PAF in effecting the release of serotonin from rabbit platelets or in inducing a hypotensive response in rats.\(^{13}\)

D-PAF, the isomeric enantiomer of L-PAF, possesses no platelet aggregating or hypotensive properties,\(^{13}\) yet it is highly cytotoxic in the HL-60 cell system (Fig. 1). In contrast to D-PAF, the D forms of the alkyl-2-lyso and alkyl-2-acyl analogs possess only slight cytotoxic activity in the HL-60 cells (Table 1). Metabolism of D-enantiomers of naturally occurring phospholipids has not been investigated in detail; however, Snyder et al.\(^{34}\) found that the D and L forms of hexadecylglycerol served equally well as a substrate for the Pte-\(\cdot\)\(H_2\)-dependent alkyl cleavage enzyme in rat liver microsomes. Our results show that significant differences in cytotoxic activities exist between the D and L isomers of PAF and between the D and L forms of alkyl-2-lyso analogs.

A sharp increase in the cytotoxic action of the 2-lyso derivatives and L-PAF on HL-60 cells was found at 10–20 \(\mu M\) concentrations of these compounds (see Figs. 1 and 2). The effects could be attributable to the detergent characteristics of each individual compound. However, Andreessen et al.\(^4\) have argued against a detergent-like effect as the basis for the antineoplastic action, as 1-alkyl-2-lyso-GPC is less effective than 1-alkyl-2-methoxy-GPC in causing cell death. Although the physical characteristics and lytic properties of several alkyl-phospholipid derivatives have been reviewed,\(^{35}\) the detergent properties of structurally related alkyl-phospholipid analogs merit further investigation.

The mechanism that accounts for the selective cytotoxic action of alkyl-phospholipid analogs in tumor cells has not been determined.\(^{12}\) One explanation for this selective antineoplastic action may be the inability of tumor cells to metabolize the ether linkage of the phospholipid due to the low activity of a microsomal Pte-\(\cdot\)\(H_2\)-dependent alkyl monooxygenase in tumors as compared to normal tissue.\(^{3,36,37}\) The relationship of the alkyl cleavage enzyme to the cytotoxic properties of 1-alkyl-2-methoxy-GPC was recently emphasized by results that showed tumor cells with the lowest enzyme activity were most sensitive to the analog.\(^{36}\) The accumulation of alkyl-phospholipid analogs in tumor cells (due to the lack of alkyl cleavage activity) could interfere with phospholipid metabolism and/or disrupt cell membrane integrity, leading to cell death. A disturbance in phospholipid metabolism is particularly likely where the 2-position of the alkyl-phospholipid contains substituents that would appear to make them metabolically stable, possibly due to the inability of such phospholipids to serve as substrates for phospholipase \(A_2\) and acyltransferases. Therefore, disruption of normal phospholipid metabolism could play a vital role in the cytotoxic mechanism of the alkyl-phospholipid derivatives. Phospholipid-sensitive Ca\(^{2+}\)-dependent protein kinase may also be a component in the antineoplastic mechanism of the alkyl-phospholipid analogs, as Helfman et al.\(^{39}\) recently reported that 1-octadecyl-2-methoxy-GPC inhibited this phosphorylation system in human leukemia cells.

The close structural similarities between \(sn-2\)-substituted alkyl-phospholipids and L-PAF suggest that the selective cytotoxic action expressed by these compounds could be mediated through an interference with some essential function(s) controlled by PAF.
Our kinetic characterization of the cytotoxic response in HL-60 cells after their exposure to the unnatural PAF analogs provides a model that can be used to explore the biochemical mechanism of action of these novel antitumor O-alkyl lipids.

ACKNOWLEDGMENT

The authors are grateful to Anne Sayer for morphological identification of the human blood cell preparations.

REFERENCES

37. Soedamsa J, Piantadosi C, Snyder F: The biocleavage of

From www.bloodjournal.org by guest on October 23, 2017. For personal use only.


Cytotoxicity of platelet activating factor and related alkyl-phospholipid analogs in human leukemia cells, polymorphonuclear neutrophils, and skin fibroblasts

DR Hoffman, J Hajdu and F Snyder