Effect of Dose, Schedule, and Route of Administration on the in Vivo Toxicity and Antitumor Activity of Two Activated Sulphhydryl Derivatives of Cyclophosphamide

Lori M. Ramonas, Leonard C. Erickson, Helmut Ringsdorf, and Daniel S. Zaharko

ABSTRACT

Two cyclophosphamide (CP) derivatives, 4-S-(hexane-6-ol)-sulfidocyclophosphamide (C-1) and 4-S-(propionic acid)-sulfidocyclophosphamide (C-2), that hydrolyze spontaneously under physiological conditions to 4-hydroxycyclophosphamide, are compared to CP for antitumor activity in male C57BL/6 x DBA/2 F1 mice with ascites L1210 leukemia or solid Lewis lung carcinoma. When C-1 or C-2 is administered i.p. as a single injection at 10% lethal dose (~LD10) to mice bearing L1210 (1 x 106 cells i.p.), early treatment produces a 5- to 6-log tumor cell kill and results in substantial numbers of long-term survivors (~30 days). Such antitumor activity is comparable to that of CP treatment. However, i.p. administration of either sulfido derivative produces liver atrophy and fibrosis of hepatic capsular structures. Hepatotoxicity is eliminated if single-dose C-2 (~LD25) is administered i.v.; however, when administered by this route, C-2 results in only a 1-log cell kill of i.v. implanted leukemic cells as compared to the 4-log tumor cell kill obtained with CP given i.v. In addition to hepatotoxicity, C-2 causes an acute and dose-limiting toxicity in mice, manifested by severe muscular spasms and cessation of breathing. In the treatment of advanced L1210, C-2 shows no therapeutic advantage over CP. When mice bearing s.c. Lewis lung carcinoma receive early i.p. treatment with CP, C-1, or C-2, each drug results in long-term tumor-free survivors. However, CP (~LD10) consistently cures all mice, whereas C-1 or C-2 (~LD10) produces only 10 to 30% tumor-free survivors. These data suggest that, in the L1210 and Lewis lung tumor systems studied, the two activated CP derivatives offer no therapeutic advantage over CP. In addition, two forms of toxicity occur with these derivatives that do not occur with CP.

INTRODUCTION

CP, a widely used drug in cancer chemotherapy (8, 13, 14), requires enzymatic hydroxylation by liver microsomes before demonstrating antitumor activity (3). Since the efficiency of hepatic mixed-function oxidases may vary considerably (19), it could be therapeutically advantageous to bypass the initial metabolic hydroxylation by delivering CP in an activated form.

The primary metabolic product of CP, the 4-hydroxy derivative, has a TI comparable to that of the parent compound (2, 11). However, 4-OH-CP is a highly unstable molecule, and it is not suitable for routine use in experimental or clinical chemotherapy studies (22).

Recently synthesized 4-alkyl-sulfido-CP derivatives are more stable crystalline compounds which hydrolyze to 4-OH-CP under physiological conditions (9, 16, 17). Since these agents are designed to be active following spontaneous hydrolysis, they circumvent the crucial reliance on enzymatic hydroxylation. Because sulfido-CP derivatives revert to 4-OH-CP in vivo, they may retain the antitumor activity and specificity displayed by 4-OH-CP.

The in vivo toxicity and antitumor activity of 2 activated sulphhydryl derivatives of CP on L1210 leukemia and Lewis lung carcinoma with respect to dose, schedule, and route of administration are presented.

MATERIALS AND METHODS

Animals and Tumors. Mice were obtained from the Mammalian Genetics and Animal Production Section, National Cancer Institute. They were housed in plastic cages in groups of 5 to 10 and given pelleted food and water ad libitum. Male C57BL/6 x DBA/2 F1, (hereafter called BD2F,) mice, weighing 20 to 25 g, were used in toxicity determinations and in chemotherapy studies with L1210 leukemia and Lewis lung carcinoma. Lewis lung was determined by comparing the median survival time of treated groups (7) with that of a control group (C) (run simultaneously in each experiment) and expressed as a percentage of ILS.

% of ILS = \((T/C - 1) \times 100\)

ILS calculations considered dying animals only; cured or long-term surviving animals (30-day L1210 survivors, 53-day tumor-free Lewis lung survivors) were noted separately (20). Tumor growth inhibition of the s.c. Lewis lung carcinoma was also used in evaluating antitumor activity. Tumor measurements...
were made 3 times a week after the tumors became palpable (~10 days following inoculation). The tumor weight was estimated by caliper measurement of the long (A) and short (B) axes of the tumor using the following formula (6).

\[
Wt \ (mg) = \frac{A \ (mm) \times B^2 \ (mm)}{2}
\]

Hepatotoxicity determinations were performed by macroscopic and microscopic examination of mouse livers removed from long-term L1210 survivors that had been treated previously with single i.p. doses (~LD_{10}) of either sulfido-CP derivative.

**Drugs.** CP (NSC 26271) and PM (NSC 69945) were supplied by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute. Each drug was dissolved in 0.85% sodium chloride solution and immediately injected into mice, either i.p. or i.v., via the tail vein.

The 2 sulfido-CP derivatives illustrated in Chart 1, C-1 and C-2, were kindly supplied by W. Klesse and Dr. T. Hirano of the Institute of Organic Chemistry, Mainz University, Mainz, Federal Republic of Germany. The C-2 acid was quite soluble in aqueous buffers of alkaline pH exceeding pH 7.4 and was administered in 0.1 M sodium phosphate buffer (pH 7.9) or 2% NaHCO₃ (pH 8.2). The C-1 alcohol, which is not very water soluble, was first dissolved in DMSO (Fisher Scientific Co., Fairlawn, N. J.), then diluted with an equal amount of Emulphor (GAF Corp., New York, N. Y.), and made up to volume with 0.1 M sodium phosphate buffer (pH 7.9) (DMSO:Emulphor:sodium phosphate, 1:1:4.7). Such a vehicle proved nontoxic to mice when given i.p. or i.v., and it allowed C-1 to be administered i.p. at high concentrations as a true solution rather than as an emulsion. Although C-1 (200 mg/kg) in 15% DMSO was nontoxic when administered i.p., it was 100% lethal when administered i.v. For this reason, true solutions of large, single i.v. doses of C-1 were not given in this study.

Acrolein and 3-mercaptopropionic acid, both from Aldrich Chemical Co., Milwaukee, Wis., were used in animal toxicity studies without further purification. Both compounds were diluted with water immediately prior to injection in mice.

All experiments, unless otherwise stated, were with single i.p. doses of drugs administered at 0.01 ml/g of body weight. Deaths were recorded daily, and body weight was recorded every 3 days following inoculation.

**RESULTS**

**Toxicity.** The relative toxicities of single i.p. doses of CP, C-1, C-2, and PM are illustrated in Chart 2. Comparison on a molar basis shows that C-1 and C-2 (at probit 3.72, LD_{10}) are more toxic than is either the parent compound, CP, or the ultimate alkylating species, PM (7). Unlike CP, both sulfido derivatives, when administered i.p. as a single injection (~LD_{10}), produce atrophy of the liver (liver weight loss, ~50%) and fibrosis of the hepatic capsules within 2 weeks of drug injection. When C-2 is given as a single i.v. injection (~LD_{10}) via the tail vein, hepatotoxicity does not occur. Single molar equivalent i.p. doses of acrolein, PM, or 3-mercaptopropionic acid (all catabolic products of C-2), either alone or in combination, failed to cause hepatotoxicity. It is likely, therefore, that the equilibrium product of C-1 or C-2 hydrolysis, 4-OH-CP, alone or in combination with its catabolic products, is responsible for this dose-dependent hepatotoxicity.

An additional toxicity, peculiar to C-2, is indicated by the different slope of the log dose-probit curve of C-2 (Chart 2). This toxicity, which is acute and dose limiting, occurs when C-2 is given i.p. or i.v. to mice, and it is manifested within 5 to 15 min by severe muscular spasms and fatal respiratory failure. Lethal amounts of C-1 do not produce this acute toxicity. Since molar equivalents of 3-mercaptopropionic acid administered i.p. or i.v. to mice are also identified as a cause, this spastic toxicity is attributed to the 3-carbon chain propionic acid moiety released by C-2.

**L1210 Leukemia Implanted i.p. or i.v.** Results from initial experiments comparing the antitumor effectiveness of i.p.-administered CP, PM, C-1, and C-2 on i.p. L1210 are summarized in Table 1. Early treatment with either sulfido derivative produces a large log tumor cell kill and substantial numbers of long-term survivors. A comparative evaluation of the therapeutic indices (1, 4) for CP, PM, C-1, and C-2 is shown in Table 2. The TI's of 2.28 and 1.79 for C-1 and C-2, respectively, indicate a relatively low margin of safety but suggest a slight advantage for C-1 over CP (TI = 1.81).

The water-soluble derivative, C-2, was studied more extensively by i.v. administration and compared with CP. The data in Table 3 show that both CP and C-2 are less active by i.v. than by i.p. administration when the tumor is implanted i.p. However, C-2 is less active than is CP by either route of drug administration at nontoxic doses. Unlike CP, C-2 loses 2 logs of antitumor activity when given i.v. to an i.v.-implanted tumor in comparison to i.p. drug administration to an i.p. tumor.

When L1210 i.p. is allowed to progress to advanced stages...
prior to drug treatment, metastatic tumor invasion of the liver is extensive (21). This metastatic involvement might be expected to depress hepatic microsomal function, and a drug requiring hepatic microsomal activation might be at a therapeutic disadvantage relative to an activated derivative. The data for CP and C-2 effects on advanced L1210 are summarized in Table 4, and they indicate no therapeutic superiority for C-2. CP retains substantial antitumor activity when administered as late as Day 4, 5, or 6 following tumor inoculation, whereas the activity of C-2 is less than that of CP and decreases with increasing tumor burden.

**Lewis Lung Carcinoma Implanted s.c.** Tumor responses of BD2F, mice bearing Lewis lung carcinoma and receiving repetitive doses of CP, C-1, or C-2 are compared in Chart 3. At doses which are 70 to 100% of their respective LD_{10}, CP is superior to C-1 and C-2 in inhibiting primary tumor growth. The times required for tumors to reach 7 g in size are 18 days for control mice, 22 days for C-1-treated mice, 24 days for C-2-treated mice, and 28 days for CP-treated mice.

Using ILS and cures as the parameters with which to measure relative drug effectiveness, CP remains therapeutically superior to both activated derivatives (Table 5). CP is active and curative on Lewis lung tumors as it is with L1210, whether the drug is administered i.p. or i.v. However, with C-2, i.v. administration of the activated drug enhances survival of Lewis lung tumor-bearing mice relative to i.p. treatment. This is the opposite of the L1210 situation.

Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Approximate fraction of LD_{10}</th>
<th>% of ILS</th>
<th>Cures</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>250</td>
<td>1.0</td>
<td>6/10</td>
<td>≤1</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.8</td>
<td>133</td>
<td>5/10</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>0.6</td>
<td>160</td>
<td>5/10</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.4</td>
<td>50</td>
<td>0/10</td>
</tr>
<tr>
<td>C-1</td>
<td>150</td>
<td>0.7</td>
<td>87</td>
<td>5/5</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>0.6</td>
<td>87</td>
<td>3/5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.5</td>
<td>80</td>
<td>2/5</td>
</tr>
<tr>
<td>C-2</td>
<td>250</td>
<td>1.0</td>
<td>30</td>
<td>9/10</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.8</td>
<td>37</td>
<td>8/10</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>0.6</td>
<td>87</td>
<td>4/10</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.4</td>
<td>88</td>
<td>2/10</td>
</tr>
<tr>
<td>PM</td>
<td>250</td>
<td>1.0</td>
<td>53</td>
<td>6/10</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.8</td>
<td>167</td>
<td>7/10</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.4</td>
<td>53</td>
<td>0/10</td>
</tr>
</tbody>
</table>

* a Single-dose i.p. administration of drug 24 hr after tumor implant.

* b Determined as described by Schabel et al. (20).

* c Some mice died before tumor-bearing controls.

Table 2

<table>
<thead>
<tr>
<th>Compounds</th>
<th>No. of nonmalignant mouse treated</th>
<th>LD_{50} (mg/kg)</th>
<th>No. of tumor-bearing mice treated</th>
<th>ED_{50c} (mg/kg)</th>
<th>LD_{50} (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>50</td>
<td>272</td>
<td>90</td>
<td>150</td>
<td>1.81</td>
</tr>
<tr>
<td>C-1</td>
<td>40</td>
<td>242</td>
<td>40</td>
<td>106</td>
<td>2.28</td>
</tr>
<tr>
<td>C-2</td>
<td>70</td>
<td>339</td>
<td>70</td>
<td>189</td>
<td>1.79</td>
</tr>
<tr>
<td>PM</td>
<td>90</td>
<td>266</td>
<td>80</td>
<td>200</td>
<td>1.33</td>
</tr>
</tbody>
</table>

* a Single-dose i.p. administration of drug to nontumor-bearing male BD2F, mice or L1210 tumor-bearing mice 24 hr after tumor implant.

* b LD_{50}, 50% lethal dose.

* c Determined by linear regression. 

Table 3

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Drug</th>
<th>Route of administration</th>
<th>Median survival of dying animals (days)</th>
<th>Cures</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1210</td>
<td>CP</td>
<td>i.p.</td>
<td>18</td>
<td>15/30</td>
</tr>
<tr>
<td>(i.p.)</td>
<td>CP</td>
<td>i.p.</td>
<td>17</td>
<td>14/30</td>
</tr>
<tr>
<td></td>
<td>CP</td>
<td>i.v.</td>
<td>15</td>
<td>13/30</td>
</tr>
<tr>
<td></td>
<td>C-2</td>
<td>i.p.</td>
<td>12 (toxic)</td>
<td>12/20</td>
</tr>
<tr>
<td></td>
<td>C-2</td>
<td>i.v.</td>
<td>7 (toxic)</td>
<td>1/10</td>
</tr>
<tr>
<td></td>
<td>C-2</td>
<td>i.p.</td>
<td>14.5</td>
<td>5/20</td>
</tr>
<tr>
<td></td>
<td>C-2</td>
<td>i.v.</td>
<td>13.5</td>
<td>4/30</td>
</tr>
<tr>
<td>L1210</td>
<td>CP</td>
<td>i.v.</td>
<td>20</td>
<td>12/30</td>
</tr>
<tr>
<td>(i.v.)</td>
<td>C-2</td>
<td>i.v.</td>
<td>11.5 (toxic)</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>C-2</td>
<td>i.v.</td>
<td>10.5</td>
<td>0/20</td>
</tr>
</tbody>
</table>

* a Single-dose i.p. or i.v. administration of drug 24 hr after tumor implant.

* b Determined as described by Schabel et al. (20).

* c Average for 2 or more groups of 10 mice each from separate experiments. S.E. within ±15%.

* d Determined as described by Schabel et al. (20).

* e Average for 2 or more groups of 10 mice each from separate experiments.

* f S.E. within ±15%.

* g See Table 1.

* h Five x 10^{6} viable L1210 cells inoculated i.v. via the tail vein on Day 0 in male BD2F, mice; median survival time for controls, ~6.5 days.

Chart 3. Inhibition of primary tumor growth in Lewis lung tumor-bearing mice treated with CP, C-1, or C-2. Male BD2F, mice were inoculated s.c. on Day 0 with 1 x 10^{5} viable Lewis lung cells. Drug treatment i.p. as indicated, began 24 hr after tumor implant. The mean tumor weights are based on 8 mice/group. S.E. are within ±15%.

Table 4

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Day of treatment</th>
<th>Median survival of dying animals (days)</th>
<th>Approximate no. of tumor cells surviving treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>200</td>
<td>4</td>
<td>16.5</td>
<td>1 x 10^{6}</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>17.5</td>
<td>7.5 x 10^{6}</td>
<td>7 x 10^{2}</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>18.5</td>
<td>5 x 10^{6}</td>
<td>7 x 10^{2}</td>
</tr>
<tr>
<td>C-2</td>
<td>200</td>
<td>4</td>
<td>15.0</td>
<td>1 x 10^{6}</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>14.5</td>
<td>7.5 x 10^{6}</td>
<td>1 x 10^{6}</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>12.5</td>
<td>5 x 10^{6}</td>
<td>4 x 10^{6}</td>
</tr>
</tbody>
</table>

* a Single-dose i.p. administration of drug on day indicated following tumor implant; doses at 0.8 of LD_{10}.

* b Determined as described by Schabel et al. (20).
to CP, Yoshida sarcoma in rats, also possessed antitumor activity. The activated derivative has greater antitumor potency than CP in the bilized, potentially allowing for transport of the active species simultaneously hydrolyze to 4-OH-CP under physiological condi-
tions, a behavior similar to a proposed protein sulfhydryl-bound derivative. The C-1 alcohol and C-2 acid, with sulfhydryl side-
chains covalently linked to the oxazaphosphorine ring, spon-
taneously hydrolyze to 4-OH-CP under physiological condi-
tions, a behavior similar to a proposed protein sulfhydryl-bound derivative. The activated analog, which would 
require drug transport by the bloodstream under these circum-
stances, does not readily reach nonlocalized tumor sites in quantities comparable to those of CP-active metabolites.

CP-activated analogs administered i.p. are not as effective as is CP in inhibiting primary Lewis lung tumor growth or enhancing the ILS of tumor-bearing mice. Since the primary Lewis lung tumor grows s.c. (15), drugs delivered i.p. must first pass through the liver before being transported to the tumor. Administration of C-1 and C-2 i.p. could result in their deactiv-
tion by aldehyde dehydrogenase on their first passage through the liver, thereby putting them at a therapeutic disad-
antage. C-2 administered i.v. circumvents the problem of first-pass hepatic drug deactivation and probably allows more of the activated species to reach remote tumor sites. This as-
sumption is suggested by the relative ILS of Lewis lung tumor-
bearing mice when i.v. and i.p. treatment results are compared.

These data from 2 tumor models suggest that differences in the transport forms of the activated analogs and activated metabolites of CP generated in vivo may account for the differences in selective antitumor action in vivo. The 4-position of the oxazaphosphorine ring appears to be the crucial site for specific transport carrier binding (23), and it is likely that the 4-
position sulfhydryl side-chain modifications of C-1 and C-2 prevent such specific protein binding. The strong binding of 4-
OH-CP to a transport carrier may serve 2 critical functions: (a) if the carrier is a macromolecule, it may prolong the biological half-life of 4-OH-CP by preventing its elimination by the kidneys or its oxidation by deactivating enzymes (5); (b) a macromole-
cule could direct the bound 4-OH-CP towards specific cells, either by attaching itself to a cell membrane or by being phagocytized by a cell, then releasing 4-OH-CP intracellularly (12). These speculative advantages may be lost with the C-1 and C-2 derivatives, and such loss may explain their decreased antitumor effectiveness in comparison to that of CP.

A great limitation with the use of these 2 sulfhydryl-CP derivatives as chemotherapeutic agents lies in their unusual in vivo toxicities. Hepatotoxicity, resulting from i.p. administration of either C-1 or C-2, cautious against the use of these activated compounds in local chemotherapy such as organ perfusion or i.p. lavage (17). The dose-limiting spastic toxicity occurring when C-2 or 3-mercaptopropionic acid is administered i.p. or i.v. suggests that 3 carbon alkylthio substituents, attached to the oxazaphosphorine ring and released during the spontaneous in vivo hydrolysis of the drug, are responsible. Despite the unusual in vivo toxicities of C-1 and C-2, these CP deriva-
tives, with their capability of bypassing enzymatic activation, should remain potentially useful for in vitro experiments inves-
tigating cytotoxicity and DNA damage by 4-OH-CP or its sub-
sequent products on the cellular level. Experiments measuring the kinetics of DNA interstrand cross-link formation by C-1 and C-2, using the alkaline elution technique, are currently being conducted.

Further analog development of these activated derivatives, with attempts to eliminate the above-mentioned in vivo toxicities, appear warranted.

**ACKNOWLEDGMENTS**

The authors thank E. Gregory and Q. Nguyen for their excellent technical assistance and Dr. B. Ardalan for the evaluation of liver toxicity. We also thank W. Kleese and Dr. T. Hirano for supplying the C-1 and C-2 derivatives.

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**Table 5**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Route of administration</th>
<th>No. of experiments</th>
<th>% of ILS*</th>
<th>Cures</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>200</td>
<td>i.p.</td>
<td>1</td>
<td>6/6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>i.v.</td>
<td>1</td>
<td>6/6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>i.p.</td>
<td>2</td>
<td>87</td>
<td>14/15</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>i.v.</td>
<td>1</td>
<td>0 (toxic)b</td>
<td>9/10</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>Days 1 to 6</td>
<td>i.p.</td>
<td>1</td>
<td>110</td>
</tr>
<tr>
<td>C-1</td>
<td>200</td>
<td>i.p.</td>
<td>1</td>
<td>125</td>
<td>2/6</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>i.p.</td>
<td>1</td>
<td>85</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>Days 1 to 6</td>
<td>i.p.</td>
<td>1</td>
<td>33</td>
</tr>
<tr>
<td>C-2</td>
<td>200</td>
<td>i.p.</td>
<td>2</td>
<td>47</td>
<td>2/14</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>i.p.</td>
<td>1</td>
<td>60</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>i.v.</td>
<td>2</td>
<td>110</td>
<td>3/16</td>
</tr>
<tr>
<td></td>
<td>31.25</td>
<td>Days 1 to 8</td>
<td>i.p.</td>
<td>1</td>
<td>33</td>
</tr>
</tbody>
</table>

* Average values used when more than 1 experiment performed. SE. within ±20%.

b See Table 1.

**DISCUSSION**

In an attempt to find CP analogs with an anticancer action superior to that of the parent compound, activated sulfhydryl derivatives of CP (C-1 and C-2) were tested in vivo for antitumor activity. The C-1 alcohol and C-2 acid, with sulfhydryl side-
chains covalently linked to the oxazaphosphorine ring, spon-
taneously hydrolyze to 4-OH-CP under physiological condi-
tions, a behavior similar to a proposed protein sulfhydryl-bound derivative. The C-1 hexanol-sulfido-CP and the C-2 propionic acid-sulfido-CP exhibit half-lives of hydroly-
sis of 9 and 8 min, respectively (10). In contrast, albumin-
bound 4-OH-CP is stable for hours (23, 24). In both instances, however, the activated 4-OH-CP metabolite is temporarily sta-
bilized, potentially allowing for transport of the active species to sites of action.

The results of the present study indicate that neither activ-
ated derivative has greater antitumor potency than CP in the 2 tumor systems examined. Other investigators (16) have found that an ethanol-sulfido-CP derivative in a tumor very sensitive to CP, Yoshida sarcoma in rats, also possessed antitumor activity like that of CP but at a TI 3 times less than that of CP.

In our experiments, when C-1 and C-2 were administered i.p. to treat an early i.p.-implanted ascites L1210 tumor, the ana-
logs demonstrated comparable therapeutic effectiveness to CP. Under these circumstances, the activated derivatives were delivered directly to the major tumor site (21). An enhanced therapeutic response probably resulted because the primary tumor mass in the peritoneal cavity was destroyed, due to high local concentrations of C-1, C-2, and their hydrolytic products, before metastasis could occur.

If the i.p. L1210 tumor is allowed to progress to advanced stages before treatment commences, substantial numbers of tumor cells metastasize and invade the marrow, spleen, liver, blood, thymus, brain, and lungs (21). When activated C-2 is used to treat advanced L1210 it becomes less effective than CP. Similarly, if L1210 is inoculated via the tail vein, thereby simulating an artificial metastatic condition in the mouse, CP is again superior to C-2 with respect to antitumor activity. Both observations suggest that the activated analog, which would
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Effect of Dose, Schedule, and Route of Administration on the in Vivo Toxicity and Antitumor Activity of Two Activated Sulfhydryl Derivatives of Cyclophosphamide

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